Monomer conversion and colour of antibacterial, remineralising Dental Composites

Submitted by

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Dedication

Dedicated to my family and friends, with special dedication to Abdullah and Safia Al Busaidi for their endless love, care and support.

Declaration

I declare that the work represented in this thesis is the result of my own investigations, except where otherwise stated. Information from the published and unpublished work of others had been acknowledged in the text and the relevant references are included.

Shaikha Al Marhubi
(Eastman Dental Institute, University College London, 2017)

Signature: ---------- Date: ----------

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Abstract

The aim of this research was to develop a composite dental material that has a higher monomer conversion by replacing the commonly used bulk monomer Bisphenol A-glycidyl methacrylate (BisGMA) with Urethane Dimethacrylate (UDMA) and by replacing Triethylene Glycol Dimethacrylate (TEGDMA) with a more biocompatible and larger diluent monomer Poly-Propylene Glycol Dimethacrylate (PPGDMA).

The study assesses the effect of including an adhesion promoting monomer 4-Methacryloyloxyethyl trimellitate anhydride (4META), and different levels of initiator Camphorquinone (CQ) and polymerisable activator2-Dimethyl amino ethyl methacrylate (DMAEMA) on conversion, colour and clarity of composite monomers.

The change in mass and volume and the final water sorption were evaluated for active composite materials that contained reminerelising monocalcium phosphate monohydrate (MCPM) and antibacterial, polylysine (PLS) and the colour change effect was assessed following immersion of the active composite discs in different solutions.

Methods: Composite monomers were prepared by mixing UDMA and either PPGDMA or TEGDMA at a 3:1 ratio. CQ & DMAEMA were added at 0.5, 0.75 or 1 wt%. All monomers contained the adhesion promoting monomer 4META at 5 wt% intially). A standard 7-µm glass was used at powder/liquid ratio (PLR) of 4.

UV-spectrometry was used to assess the colour and clarity of the monomers. FTIR (infrared) was employed to determine monomer conversion (MC) with 40s light exposure at 1 mm depth.

With the elimination of the activator DMAEMA, clear UDMA/PPGDMA monomers that also contained 3 wt% 4META were used with hybrid glass, MCPM and PLS to contract composite discs that were used to check mass and volume changes at 37 C^{o}.

The effect of extrinsic discolouration of the experimented MCPM and PLS containing composites was also evaluated visually and using a colour measuring instrument (spectroshade). Values of colour change were recorded in the form of ΔE and the change was compared to a commercial material Z250.

Results: Composites prepared with PPGDMA had approximately 20% higher conversion. DMAEMA resulted in increased clarity of the monomers by enhancing

dissolution of 4META as demonstrated by UV spectrometry.
The addition of DMAEMA had no significant effect on MC of monomers containing PPGDMA but increased MC in those containing TEGDMA. CQ concentration enhanced colour. Maximum conversion was obtained with 0.75 wt% CQ in UDMA/PPGDMA, 3 wt% 4META composites. Active composites with PLR 3 and 5 wt% of PLS resulted in the highest percentage in mass change with more than 4%. Higher volume changes were obtained with formulations of PLR 4 and 5 wt% PLS.
There was no significant difference on effect of extrinsic discolouration of composite discs when visually assessed, however, using the Spectroshade it was clear that composites with PLR 5 were discoloured the most.

Conclusions: Monomer conversion can be improved by changing the monomer content of dental composites. This will result in a reduction of leaching of unreacted monomers into the oral cavity and therefore improve biocompatibility. Composites containing PPGDMA had a better monomer conversion compared to those with TEGDMA. In this research, the main intrinsic factor that affected the colour of the monomer was the concentration of CQ whereas the effect of extrinsic discolouration can be attributed to PLR.

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Abbreviations

WHO	World Health Organisation
ECC	early childhood caries
GIC	glass ionomer cements
RMGIC	resin modified glass ionomer cements
BisGMA	Bisphenol A-glycidyl methacrylate
UDMA	Urethane Dimethacrylate
PPGDMA	Poly-Propylene Glycol Dimethacrylate
TEGDMA	Triethylene Glycol Dimethacrylate
4META	4- Methacryloyloxyethyl trimellitate anhydride
NTGGMA	N p-tolyl glycidyl methacrylate
DMAEMA	2-Dimethyl amino ethyl methacrylate
DMPT	N,N-dimethyl-p-toluidine
CQ	Camphorquinone
PLR	Powder liquid ratio
PLS	Polylysine
FTIR	Fourier Transform Infrared
MCPM	Monocalcium calcium phosphate monohydrate
SDF	Silver diamine fluoride
FDI	World dental federation

Chapter one

Literature review and introduction

1 Dental caries, aetiology and management

1.1 Dental caries epidemiology and aetiology

Dental caries is an infectious disease affecting the tooth tissues, namely enamel, dentine and cementum. According to the WHO (World Health Organisation) the first global map with data on the prevalence of the disease was formulated in 1969. Dental caries can result in severe pain and can prevent a person from practicing normal daily activities e.g. attending work or school, thus affecting life style and quality. Despite the fact that, dental caries is a preventable disease, it is still one of the most common diseases worldwide and the prevalence of untreated dental caries in permanent teeth is said to be the highest if compared with other conditions (Marcenes et al., 2013).
It is important to maintain good oral health and in particular maintaining healthy teeth as current research shows correlation between healthy teeth and maintaining a good general health. For example, a study showed that children with caries have a reduced weight compared to their peers who have no caries (Sheiham, 2005).

1.2 Epidemiology

The WHO states that 60-90 percent of young people in industrialised countries have decay (Iheozor-Ejiofor et al., 2013) and almost 100 percent of adults can have the carious cavities at some life point. In an epidemiological review study carried out at the University of Michigan the author concluded that there is an increase in the prevalence of caries in several countries, and this may result in a global disaster in public health (Bagramian et al., 2009, Marcenses et al., 2013).
In addition, according to the latest results from Children's Dental Health Survey for England, Wales and Northern Ireland, it is estimated that 31% and 46% of five and eight years old respectively, are affected by caries (Vernazza et al., 2016). Twelve percent (12%) of three years old have dental caries according to the latest NHS Dental Epidemiology Programme reports for England (NHS DEP, 2013). Additionally, dental caries is one of the most common reasons why children are hospitalised (More and Ashley, 2009). Moreover, it is estimated that, globally about 35% of the population have untreated dentine caries in their permanent dentition (Marcenses et al., 2013, Frencken et al., 2017). Untreated dental caries is amongst the 100 other conditions that can be regarded as a cause of disability, in other words, adversely affecting a person's day to day activities (Marcenes et al., 2013).

1.3 Aetiology

Acids produced by bacteria, mainly *Streptococcus mutans*, and others, as a result of sugar fermentation, cause caries. These acids act by reducing the pH in the oral cavity resulting in a disturbance of the total net gain or loss of mineral i.e. the equilibrium is shiftted. This results in a process known as demineralisation, which is the route by which tooth hard tissues lose minerals. Whereas remineralisation is the process in which the minerals are driven back into the tooth surface and hence, incorporated into the tooth structure.

In the past, three hypotheses were suggested regarding how bacteria were involved in dental caries. First, the specific plaque hypothesis, in which Lactobacilli was regarded as the main causative agent (Beighton, 2005). It soon became clear that this bacterium is not the main causative factor when experiments were done on germ-free rats (Beighton, 2005). The second hypothesis is the non-specific plaque hypothesis; this states that several bacteria are involved in the process. The third hypothesis, which is the Ecological Hypothesis, is the one, which fits with complexity of the whole process (Takahashi et al, 2011).

The ecological hypothesis states that the acidic environment produced in the oral cavity by the microflora can have a role in determining the type of bacteria present (Takahashi et al, 2011). When sugar is moderately consumed; bacteria such as Actinomyces produce acids and the oral environment is said to be acidogenic. If there is, however, a prolonged and constant acid production; the result is a further reduction of the pH. The acidic environment then leads to a process in which bacteria such as *Streptococcus mutans*, which are acid producers, selectively survive (Takahashi et al, 2011).

1.4 Management of Dental caries

Dental caries is a preventable disease and management should always include preventive advice and preventive measures. Current recommendations by the Department of Health in England include the use of fluoridated toothpastes from an early age, children as young as three can use toothpaste with up to 1500 ppm of fluoride.

For young children, supervised tooth brushing as well as controlled diet and reduced frequency of sugar consumption are the main recommendations. Additionally, where a child requires medications it is advised that they are given sugar free medicines.

Dental visits should start at an early age, so that children at high risk of caries can be identified and disease can be controlled. The application of topical fluoride varnish of

22,600 ppm is one of the main and important modes of preventing dental caries; therefore, considerations should be taken particularly with high risk children as advised by the Department of Health.

Other methods include the use of casein proteins that contain calcium phosphate to aid remineralisation of early decay; these are normally presented as mousses, and may be useful for young patients who undergo orthodontic treatment.

The role of a dental team is to identify those who are at risk and develop a preventive plan. It is well known that those who have caries at an early age are at higher risk of developing caries later on. Other risk factors include poverty and socio-economic status.

As well as individual based prevention plans, caries prevention can be community based. The WHO has been working over the years in developing several policies to prevent caries and encouraging authorities in different counties to reinforce knowledge of the disease and how to prevent it. However, in countries with poor community based preventive programmes, the prevalence of caries is on the rise (Iheozor-Ejiofor et al., 2013). Brushing teeth with toothpaste that contains fluoride is a simple way of preventing caries, as it is well established that fluoride supplied in constant low levels is effective (Dos Santos et al., 2013).

Water fluoridation has been a topic of huge controversy worldwide. The WHO has, therefore, published a document on standards and guidelines with regard to water Fluoridation. The document suggests that when considering water fluoridation other sources of Fluoride should be taken into account to prevent any possible side effects.

Additionally, dental tissue lost from caries cannot be replaced; therefore a lot of effort has been made in an attempt to minimise hard tissue distruction. Modern dentistry is directed towards minimising hard tissue removal and sealing incipient carious lesions

As soon as permanent first molars erupt they should be protect with fissure sealants. This can be done using a number of dental materials depending on the situation.

A Cochrane systematic review concluded that resin based fissure sealants are effective in caries reduction for up to two years after placement (Ahovuo-Salorantaet al., 2013) and more effective than fluoride in preventing fissure caries (Ahovuo-Saloranta et al., 2016).

1.5 Other approaches to manage caries

The use of Silver diamine fluoride (SDF) has been advocated as a way of managing or arresting caries non-invasively (Sharma and Puranik, 2015, Horst and Seto, 2017). It is regarded as a safe and efficient way of managing caries by the US Institute of

Medicine, although its mechanism of action is still unclear (Rosenblatt et al, 2009, Zhao et al., 2017).

Several concentrations have been used mainly in Japan and china ranging from 12 to 38% (Zhao et al., 2017). SDF is an alkaline solution made of silver and fluoride ions, that is colourless and forms an ammonia complex (Zhao et al., 2017). Generally, despite the promising effectiveness of SDF products, there is no standard concentration of the material that has been agreed on; additionally the change in colour of the tooth surface may not be appealing to many patients.

1.6 Restoring decayed teeth

Once teeth are affected by dental caries, it is important that they are managed properly. This will depend on a number of different factors including the tooth affected, age of the patient, available materials and the clinician's choice and patient's preferences.

Untreated caries may result in severe pain and tooth loss eventually. Adding to that, children with untreated caries may experience irritation and sleep disturbance (Abanto et al., 2011), and this may affect their general performance.

1.7 Current restorative materials

1.7.1 Amalgam

Amalgam is a dental material that is made of a mixture of metals. One of the metals used is mercury combined with silver (Mccabe and Walls, 2013). Once set, amalgam is a very strong filling material and can last for several years.

Over more than 150 years dental amalgam has been used as a filling material (Rasines Alcaraz et al, 2014). This is because of the several advantages of this material. It is often referred to as the forgiving material, because moisture control when placing amalgam is not regarded as an issue unlike other materials. Additionally, it is easy to handle and working time is normally regarded as adequate.

However, since it contains mercury concerns have been raised over the safety and biocompatibility of this material. The Minamata disaster in Japan that resulted from mercury containing wastes caused a lot of concerns with regard to amalgam. The world dental federation (FDI) has been supporting the development of new alternative materials. Additionally, according to the FDI, fifty five countries have approved the convention that is to come into force by August 2017.

Other problems with dental amalgam include, the need for cutting the tooth in a specific way so that the filling is retained i.e. undercuts; resulting in sound tooth tissue being lost

unnecessarily. This is due to the fact that amalgam lacks the ability to bond to tooth tissues namely enamel and dentine, while the general trends in recent years is to preserve tooth structure (Cocco et al., 2015). Moreover, the filling often results in tooth discolouration and becomes unsightly.

1.7.2 Glass ionomer cements and other materials

Glass ionomer cements were developed in the early 1970s and were originally derived from silicate and poly-carboxylate cements (McCabe and Walls, 2013).
They bond directly to the tooth surface and do not normally require an adhesive system or a bonding agent (Selmovic-Dragas et al., 2012). They are mainly supplied in the form of powder and liquid but are also available in capsules that use an amalgamator for mixing. The powder is normally sodium alumino-silicate glass (McCabe and Walls, 2013). Glass Ionomers are known as the fluoride releasing materials; the effect is mainly through releasing of fluoride when the intra-oral pH declines (Mayanagi et al., 2014). Glass Ionomers are normally used where proper teeth isolation cannot be achieved for a resin restoration in high caries risk patients.
The other advantage of GIC is the ability to bond to tooth structure. The uses of glass ionomers are several and mainly depend on factors such as the type of filler used and the area to be restored. They can be used as fissure sealants, lining material or luting cements. However, these materials tend to have poor mechanical properties (Xu and Burgess, 2003).

1.7.3 Resin modified glass ionomer cements

Resin modified glass ionomer cements (RMGIC) were developed in the early 1980s (Cattani-Lorente et al., 1999) resulted from combining the conventional GIC with resin in order to improve some properties such as durability (Selmovic-Dargas et al., 2012). Although some studies showed that the polymerisation reaction in RMGIC is fast and takes place before the slow acid glass reaction (Young et al., 2004) other studies showed the opposite, i.e. setting reaction of RMGIC is a two-stage reaction, first the acid base setting, as the case with conventional GICs, then the polymerisation reaction when light cured (Cheetham et al., 2014).
The ability of the clinician to control the reaction is one of the main advantages of RMGIC. However, since they contain resins, RMGICs undergo polymerisation shrinkage similarly to composite resins. The resin used is often 2-hydroxyethyl methacrylate (HEMA), that if unreacted can leach out into the cells of dentine and pulp (Czarnecka et al., 2014).

1.7.4 Compomers (Poly acid modified resins)

Compomers were developed in the early 1990s (Nicholson, 2007). Compomers (also known as polyacid modified composites) are a combination of composites; (see Figure 1.1) and glass-ionomers. Polymerisation is initiated by light cure and triggering free radicals as in resin composite materials. This is then followed by a water sorption induced acid base reaction as with GIC (Sakaguci et al., 2012)

Compomers are normally supplied in pre-mixed capsules and the setting reaction is by polymerisation initiated by blue-light similar to that of composite. Although compomers resemble composites in many aspects, they are less resistant to tensile stress and are therefore more prone to fracture and their use is therefore restricted to non-stress bearing areas (Nicholson, 2007).

1.7.5 Composites

The increased demands for dental aesthetics led to the development of dental composites in the late 1950s and early 1960s. Dental composites are tooth coloured filling materials. Several features of composites make them preferred by patients and clinicians.

There are several advantages of using dental composite as a filling material. Firstly, they require minimal cavity preparation (Mehdawi et al., 2013) and so they are said to be conservative materials. Secondly, they can be bonded to tooth surfaces using modern adhesive systems. The longevity of composite materials has improved nowadays, showing satisfactory results after eight years of placement (Frankenberger et al., 2014). Composites consist typically of a resin matrix and inorganic fillers normally in the form of glass of different size (Khan et al., 2015) and a coupling agent. Manufacturers nowadays supply the material pre-mixed in tubes or syringes. Composites can be classified according to filler size for example, macrofill composites, which are conventional and typically contained filler particles of 10-50 micrometer in diameter (Shah et al., 2014) and nano-composites with particles size ranging up to 5 nano-meter (Mitra et al., 2003).

A hybrid composite would have a mixture of filler sizes as suggested from the name. Dental composites can also be classified according to the proposed use. Fissure sealants, flowable and posterior composites are examples. Flowable composites are also known as low filled composites and are known for being able adapt to the surface of the tooth (Shah et al., 2014). Another classification of composites can be according to the setting method as in self-cured or light-cured. The addition of fillers into the resin matrix of composites has contributed to some of their desirable properties, for example,

rigidity, hardness, strength and a lower shrinkage (McCabe and Walls, 2013).

Although there have been a lot of improvements in bonded dental materials such as composites, they still have poorer longevity compared to amalgams and the clinical life span of these materials is regarded as short (Moretto et al., 2012).

Direct composite materials are mainly based on a mixture of high molecular weight monomers such as BIS-GMA, a highly viscous monomer that requires other monomers such as TEGDMA as a diluent monomer (Ferracane, 2011). However, concerns were raised over the safety of some monomers as it is thought that unreacted residuals can be released into the oral cavity and result in cytotoxicity (Van Landuyt et al., 2007).

In light-cured composites, the setting reaction is initiated by a light source of blue light (460-470 nm). The higher the molecular weight of a monomer, the better, as this helps in minimising polymerisation shrinkage. Polymerisation shrinkage results in creating stress at the interface between the filling and cavity walls and is regarded as the primary reason for restoration failure (Shah et al., 2014, Tinca Buruiana, 2009). This is because a micro-gap forms when the material shrinks and this enables passage of bacteria from the oral cavity.

The second most common reason for composite failure is material fracture. This can happen because of low flexure stress resistance.

Although presently most composites are light-cured, self cured and dual cured materials are still of great use, particularly when the use of light cure is difficult as the case in core build up materials as in post-core systems (Sideridou et al., 2012). Photoinitiator systems are normally incorporated into the composite materials. Camphoroquinone (CQ) combined with an amine co-initiator is a typical example of such systems or materials that acts as an initiator of the polymerisation reaction by producing free radicals once activated by the light cure.

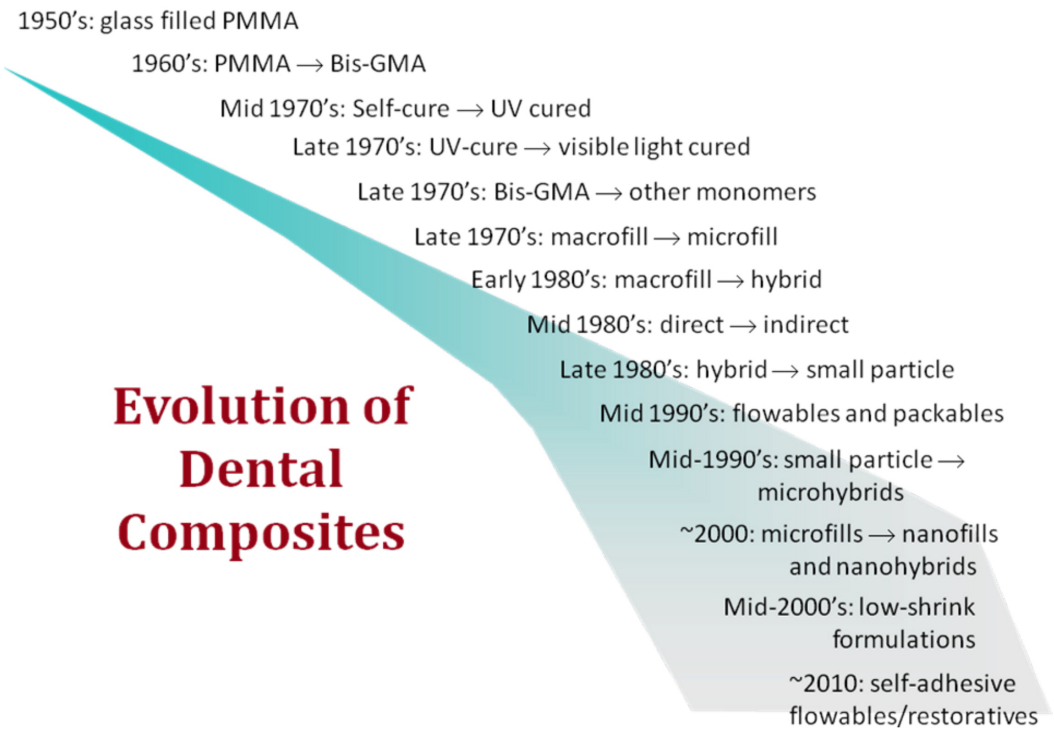


Figure 1.1 the development of dental composites (Ferracane, 2011)

1.8 Dental composites and aesthetic demands

There are several situations in which dental composite materials are required in order to restore aesthetics as an alternative to porcelain in young patients. These include enamel and dentine defects and anomalies such as dentinogenesis imperfecta and amelogenesis imperfecta.

Other developmental defects include fluorosis where teeth can be severely affected and discoloured in which case the enamel of a tooth is very weak, discoloured and brittle when fluorosis is classified as severe. Additionally, where there are anomalies of tooth size as the case with peg shaped lateral incisors, composite filling materials are used to mask the deformity.

In general, the choice of a material will depend on the clinical scenario. There is no one material that is ideal (See Table 1-1).

An ideal material should have all the favourable characteristics of the different dental materials. Dental composites being tooth coloured and strong could be the preferred material. In a systematic review it was found that only 2.4% of posterior composites fail within ten years (Opdam et al., 2014) indicating a good longevity. However, this is subject to several factors including the size and position of the cavity as well as case selection. The author stated that the risk of failure increases by up to 40% where the

carious lesion is large and involving multiple surfaces (Opdam et al., 2014). In contrast, Boaro et al stated that the longevity of composites particularly for posterior teeth is still uncertain.

The current project focuses on developing a new composite material with an improved monomer conversion, because of the improved mechanical and physical properties associated.

Material	Advantages	Disadvantages
amalgam	- mechanical properties are superior - easy handling - not sensitive to moisture - mechanical retention - excellent longevity - low cost	- poor aesthetics - not conservative - contain mercury
GIC	- realising fluoride - chemical bonding - conservative	- poor retention - aesthetics not as composite - compromised mechanical properties -poor longevity
RMGIC	- realising fluoride - controlled setting reaction - dual curing - conservative	- polymerisation shrinkage - compromised mechanical properties -longevity is compromised
compomers	- good aesthetics - good mechanical properties but not as good as composite	- polymerisation shrinkage - sensitivity to moisture - handling difficulties
composites	- excellent aesthetics - good mechanical properties - conservative -controlled setting reaction	- polymerisation shrinkage - do not bond to the tooth -handling may be difficult

Table 1-1 common dental materials and their advantages and disadvantages (McCabe and Walls, 2013).

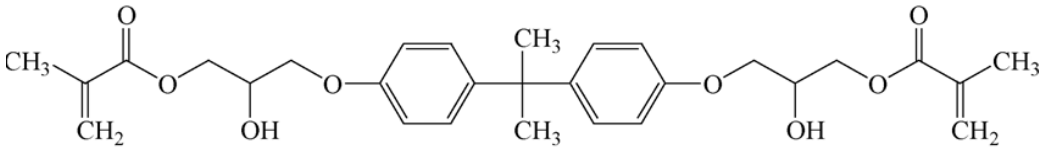
1.9 Common components used in a dental composite system:

As mentioned earlier, the main components of dental composites are: a polymeric matrix and inorganic filler coupled with a silane. Below are examples of commonly used monomers.

1.9.1 Monomers

1.9.2 Bisphenol A-glycidyl methacrylate (BisGMA) $C_{29}H_{36}O_8$

BisGMA is a highly viscous and bulky resin containing two aromatic rings and is prepared from bisphenol A and glycidyl methacrylate. It was one of the monomers to be prepared by Bowen in the late 1950s (Asmussen and Peutzfeldt, 1998). It is one of the most used dimethacrylates in commercial materials (Buruiana et al., 2009). Its high molecular weight (512g/mol) resulted in the production of stronger resins with lower polymerization shrinkage. It is mainly used as a bulk monomer and often needs to be diluted using other monomers with less viscosity (Peutzfeldt, 1997).

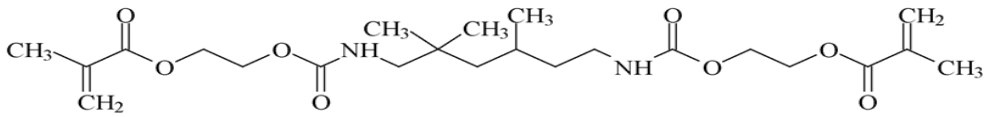


Bis-GMA

Figure 1.2 The chemical structure of Bis-GMA (Floyd et al., 2006)

1.9.3 UDMA (Urethane Dimethacrylate) $C_{23}H_{38}N_2O_8$

UDMA is used as a bulk monomer, it contains reactive carbon double bonds at each end, which means addition and crosslinking polymerization reactions can take place when the polymerization process is initiated. It is an aliphatic monomer and has a molecular weight of 470.563 g/mol, that is almost equivalent to that of BisGMA making it a good alternative to it. It is also less viscous and more flexible making it possible to be used on its own without necessitating a diluent monomer (Peutzfeldt, 1997).



UDMA

Figure 1.3 the chemical structure of UDMA (Floyd et al., 2006)

1.9.4 TEGDMA (Triethylene Glycole Dimethacrylate) $C_{14}H_{22}O_5$

TEGDMA is another monomer that is commonly used in many commercial composite restorative materials. It is often used to improve the flowablity. When viscosity is reduced more filler can be incorporated into the composite material (Barszczewska-Rybarek, 2014) thus improving some properties. This monomer has a molecular weight of 286 g/mol which being relatively low causes high shrinkage.

1.9.5 PPGDMA (Poly-Propylene Glycol Dimethacrylate) $H_2C=(CH_3)CO\ (OC_3H_6)_n\ O_2CC\ (CH_3)=CH_2$

PPGDMA is a newer alternative diluent monomer that can be used in combination with BisGma or UDMA to control viscosity. It has a molecular weight of 600 Da which is more than twice as much as that of TEGDMA (molecular weight ~ 286 g/mol) Both TEGDMA and PPGDMA are flexible monomers and contribute to cross-linking during polymerisation. The higher molecular mass than TEGDMA can improve biocompatibility. Additionally, monomers with fewer double bonds are preferred as it means they are less likely to shrink (Walters et al., 2016).

1.9.6 4META (4- Methacryloyloxyethyl trimellitate anhydride) $C_{15}H_{12}O_7$

4META is a solid monomer that is available as a powder and widely used in adhesion systems (Van-Landuyt et al., 2007). Examples of commercial materials containing 4-META are Orthomate Super-Bond and Super-Bond C&B both introduced in Japan. These were developed for bonding orthodontic brackets (Chang et al., 2002).

1.9.7 Photo-initiator systems

Initiators form radicals when exposed to certain conditions e.g. blue light, which then initiates a polymerisation reaction. The concentrations of the photoinitiator as well as the tertiary amine are important factors for monomer polymerization. It is well known that optimising polymerisation is essential for an improved colour, mechanical properties, stability and biocompatibility (Moon et al., 2012). One of the main features of photointiators is their absorption spectrum that is preferred to be within the visible light range (Van-Landuyt et al., 2007). Their absorption should correlate with the wavelength of the emitted light that is used for curing.

1.9.8 Camphorquinone (CQ) $C_{10}H_{14}O_2$

CQ is one of the most used photointiators (de Oliveira et al., 2015). It is present as a powder that is yellow in colour, at room temperature (Van-Landuyt et al., 2007). It is often combined with an amine co-initiator (de Oliveira et al., 2015) to trigger free radicals e.g. DMAEMA (2-Dimethyl amino ethyl methacrylate) or NTGGMA (N p-tolyl glycidyl methacrylate). The peak absorption of CQ is around 470 nm.

The intense yellow colour is one of the disadvantages of CQ. Although a small amount is normally used (0.1-1 wt%) (Van-Landuyt et al., 2007) it may affect the colour of the final restoration (de Oliveira et al., 2015). This issue can be reduced, however, by photobleaching

A free radical results from a reaction between the CQ and a hydrogen ion from the amine. Schneider at el demonstrated that the ideal CQ concentration is about 1-1.5 wt% used in combination with amines such as phenyl-propandeione (Schneider et al., 2009) in Bis-GMA and TEGDMA containing composites. The co-initiators used with the CQ are often linked to some tissue toxicity; thus a lot of effort is made by researchers to develop new or alternative systems (Carmer et al., 2011). Photointiators such as: α diketone PPG "(1-phenyl 1,2-propandione)" has been used in some types of composites as alternatives to CQ (Shah et al., 2009).

1.9.9 Co-initiators examples

DMAEMA ($C_8H_{15}O_2N$) (2-Dimethyl amino ethyl methacrylate) with a molecular weight of 157g/mol is one of the most commonly used activators or co-initiator.

Co-initiators do not interact with light but they interact with the excited state of a photoinitiator know as the "triplet-state", This results in the formation of a state complex or "exciplex" (Schneider et al., 2009, Musanje et al., 2009). Different concentrations of this amine can be used together with CQ to achieve a desirable monomer conversion (Yoshida and Greener, 1993).

Examples of other activators often used together with CQ are DMPT (N,N-dimethyle-p-toludine), and NTGGMA (N p-tolyl glycidyl methacrylate).

NTGGMA is a solid amine accelerator that also acts as an adhesive monomer. Furthermore, it has a methacrylate group that can react with the free radicals produced during the process of polymerisation. This may help prevent release of this amine and alsocontributing to bonding of a material to tooth structure.

1.9.10 Other initiators

An example of an initiator system that is commonly used in self-cured composites is Benzoyl Peroxide (BPO), together with an aromatic tertiary amine activator (Sideridou et al., 2012).

1.10 Glass and inorganic fillers

Inorganic fillers are used to create bulkiness or to give body to the composite material. The addition of fillers plays an important part in the determination of some properties of the final material (Silikas et al., 2007). Glass in the form of quartzor mineral silicates is commonly used at different concentrations (Silikas et al., 2007). There are different sizes of glass that can be used. For example, the bigger parts are macrofill and size range is 10-15 (μm) while microfill particle size range from 40-50 (μm) and the nanofill range from 5-100 (μm) (Ferrancane, 2011).

1.10.1 Hybrid composites

When the different sizes of glass particles of are used the composite is said to be a hybrid (Silikas et al., 2007). The shapes and sizes of glass particles can enhance some features such as adaptation of the material within a cavity (Shah et al., 2014) and perishability that is important for aesthetics (Silikas et al., 2007). In addition, polymerisation shrinkage may increase when smaller partiicles are used.

1.11 Coupling agents

In a dental composite system coupling agents are normally used to create a bond between the organic and inorganic parts (Halvorson et al., 2003). A recent review study stated that there are several researches indicated that there is an increase on bonding strength when particles are silanated (Spitzangel et al., 2014).
Titanates and Zirconates are examples of coupling agents, however the most commonly used coupling agents are the organic silanes (Anusavice et al., 2013) such as γ-methacryloxypropyl.

1.12 Common problems with dental composite

1.12.1 Recurrent caries

The most common reason for replacement of a restoration is recurrent caries (Antonucci et al., 2009, Ferracane, 2011). With regard to composite dental filling materials, recurrent or secondary caries is widely linked to polymerisation shrinkage

(Shah et al., 2014) and caries (Aljabo et al., 2015, Liu et al., 2013).

1.12.2 Wearing of dental composite materials

While most patients as well as clinicians prefer tooth coloured restorations, the greatest challenge of developing a material as strong as amalgam yet tooth coloured remains a controversial issue. Wear resistance is one of the most important mechanical properties of a composite material that is to be used on a posterior tooth. Increasing filler content may help in improving physical properties. Physical properties are affected by several factors. For example, monomer conversion and type of filler used. The higher the monomer conversion, the better the physical properties of a composite material (Sideridou et al., 2012).

Filler sizes have changed dramatically over the years. Early composites had filler particles that range from 10 to 50 μm (Mitra et al., 2003), in an attempt to improve physical properties and reduce shrinkage. Currently, as there are demands for highly polished and smooth composites yet with improved physical properties; particle sizes have been reduced to submicron sizes.

1.12.3 Strength and stress resistance under masticatory forces

Flexure strength is defined as the ability of the material to resist fracture under stress (Chung et al., 2004). This can be determined using a “ball on ring” jig using a mechanical frame produced by companies such as Instron.

Elasticity of a material indicates its ability to withstand deformation under stress. Depending on the surface to be filled, the importance of both properties varies. Therefore for posterior restorations, where masticatory forces are high, higher flexure strength and elasticity are desirable.

1.12.4 Polymerisation shrinkage

Polymerisation shrinkage is still one of the major challenges that manufacturers are faced with. This is because it results in changes in the interface between the material and the tooth surface. The result of polymerisation shrinkage is a gap formation, filling fracture or tooth fracture reducing the longevity of the restoration. In all three scenarios, the end result may be costly and psychologically damaging for the patient. One of the first signs that a patient may experience is post-operative sensitivity and discomfort (Shah et al., 2014).

Polymerisation shrinkage results in stress build up within the composite material (Boaro et al., 2013, Shah et al., 2014). The stresses generated are also transferred to

the adhesive and the tooth structure (Shah et al., 2014). The amount of stress depends on the shrinkage and modulus of the composite (Abu-eleniain et al., 2013) and correlates with monomer conversion ((Sideridou et al., 2012).

1.12.5 Monomer conversion MC

Monomer conversion is normally calculated in percentage and it reflects the amount of carbon-carbon double bonds converted into single bonds (Liaqat, 2015). It affects several properties including mechanical properties and polymerisation shrinkage (Leprince et al., 2013). It is normally assessed using FTIR (Fourier Transform Infrared). This is an instrument that uses attenuated total reflected infrared beam that hits a solid or liquid sample. The detector is connected to a computer that uses specialised software. The spectrum is then displayed on a monitor as a graph, from which the degree of monomer conversion can be calculated. However, there are several other options for checking or determining the degree of conversion for example, some indirect methods that depend on determining the micro hardness differential thermal calorimetry (DCT) or defferential scanning calorimetry (DSC) (Santan et al., 2009, Liqat, 2015).

Monomer conversion depends on several factors; such as the chemical structure of the monomer mix, temperature and the concentration of the activator used (Sideridou et al., 2012). Another technique used to check monomer conversion is Raman Spectrometry that is used to quantify the amount of unreacted monomers similar to FTIR (Liaqat, 2015). Where the degree of conversion is low, unreacted monomers can leak out of the material leading to compromised biocompatiability and mechanical properties (Du and Zheng, 2008).

Several factors can affect the degree of monomer conversion, for example, the nature and chemistry of the monomers, type of initiators and inhibitors if used as well as fillers concentrations and the type of light cure (,Liaqat, 2015, Peutzfeldt and Asmussen, 2005).

1.12.6 Techniques to reduce microleakage

Microleakage is the process by which bacteria gains access into the gap between a tooth and the restorations. The main cause of microleakage is polymerisation shrinkage. It results in recurrent or secondary caries and failure of a restoration. The end outcome may be severe pain and loss of tooth vitality. In worst cases the tooth is rendered unrestorable and therefore requires an extraction. Several approaches have been introduced in order to overcome this problem. These include, manipulation of

curing technique and curing time, changing of monomers used within a composite organic matrix and changing the polymerisation mechanism (Carmer et al., 2011).

One of the main techniques is the use of a liner or a base. Glass ionomer cements have been used widely as liners where a composite filling material is used to restore a tooth. Another technique to avoid microleakage is to minimise polymerisation shrinkage. This can be achieved by incremental placement of a composite material and curing at different angles. Additionally, placement of composite in small increments will insure that correct depth of curing is achieved.

When a higher filler content is used in a certain material this means that less monomer is required, therefore minimising the shrinkage and micro-gap formation.

Moreover, some dental adhesives, particularly the ones containing acidic monomers may exhibit some antibacterial effect for the first two days after placement (Cocco et al., 2015)

This, however, may help in minimising the bacterial load for a short period but will not decrease staining of the filling. Researchers are working more towards the incorporation of antibacterial substances into the filling materials.

1.12.7 Other characteristics of dental composites

1.12.7.1 Water sorption

Diffusion of water in to the composite material is known as water sorption (Örtegren et al., 2001). It is a feature that results in the expansion of the material, which may be useful to compensate for polymerisation shrinkage (Versluis et al., 2011). It can be induced by the incorporation of elements that can leach out of the composite material such as Mono Calcium Phosphate Monohydrate (MCPM) (Wei et al., 2013, Panpisut et al., 2016). The amount of water sorption varies between different materials, if water molecules are incorporated inside the composite matrix, this may attract the absorption of more water molecules, which may disrupt hydrogen bondings within a material and compromise some of the mechanical properties (Liaqat, 2015) as a result. For example, excessive expansion of a material can result in internal stresses on the tooth that in turn will result in tooth fracture (Watts et al., 2000, Liaqat, 2015).

1.12.7.2 Colour instability

Colour is basically a different form of visible light. When a white light beam is projected on a glass prism the result is a dispersion of white light into the different colours. The human eye perceives colour by analysing the spectrum of visible light and stimulating

the colour receptors in the retina then to the brain through the optic nerve (Johnston, 2009).

1.12.8 Light and colour (reflection and scatter)

Visible light is a range of different frequencies combined together (Shapley, 2012). Norman Campbell a scientist during the early twentieth century was one of the first to attempt quantifying light (Johnston, 2015). The science of colour measuring is referred to as spectrophotometry (Plataniotis and Venetsanopoulos, 2013). The colour of an object can only be detected based on light absorbed and scattered (Shapley, 2012).

1.12.9 Colour Matching

More patients than ever are demanding tooth coloured restorations, the increasing demands and expectations cauase concern for both patients and clincians (Khan et al., 2015). Several factors have to be taken into account when trying to match a filling material with the patient's tooth, these include not only the colour of the patient's teeth but also the skin colour of a person, age and occupation, and in terms of the tooth being treated it also depends on the degree of glaze and surface texture (Stevenson, 2009).

Numerous studies demonstrated the factors affecting composite colour (Ardu et al., 2011). These factors can be devided into intrinsic and extrinsic (Catelen et al., 2011), For example, the monomer composition and the degree of monomer conversion (Shah et al., 2014), may affect colour stability. Other factors include the filler used in terms of size and nature (Ardu et al., 2011) and the type of photointiator and accelerator used (Luiz et al., 2007). Food and drinks are regarded as extrinsic causatives of composite fillings discolouration.

A lot of knowledge in colour is required as suggested by Stevenson in his review on methods of colour matching (Stevenson, 2009). In 1905 Albert H. Munsell developed the first system description using three important factors, the hue, the value and the chrome (Pustina-Krasniqi et al., 2015).

1- The Hue: Describes the colour group itself, for example, yellow or brown etc.
2- The value: This can be referred to as how much light is reflected by an object to appear dark or light. Therefore, a material with a high value reflects more light
3- The Chroma

Chroma refers to the quality of the hue and its saturation (Pustina-Krasniqi et al., 2015).

As enamel is translucent most of the tooth colour is mainly because of the dentine, thus in older individuals teeth appear dark, due to tertiary dentine formation. Although shade selection is subjective, it can be affected by several factors such as, type of light e.g. daylight or artificial light, shade guide used and probably colour of the surrounding environment.

1.13 Methods of colour measuring

Colour matching is still a great challenge in dentistry. The main goal of measuring the shade is to reproduce aesthetics (Johnston, 2009). A lot of research has been carried out on colour in dentistry over the past few years (Chu et al., 2010). Colour instability is one of the reasons why composite fillings are replaced (de Oliveira et al., 2015).

When it comes to shade selection; the most commonly used method is the direct visual method with the use of shade guides such as the Vita system (Chu et al., 2010). There are some problems that are associated with or can affect the use of visual systems; for example, the quality of room light may affect the final shade being selected as well as the degree of eye fatigue and physiological differences of the person selecting the shade (Chu et al., 2010, Kim-Pusateri et al., 2009). All of the above mentioned problems result in visual colour assessment being subjective even under ideal conditions (Kim-Pusateri et al., 2009).

Additionally, there is a phenomenon known as metamerism that greatly affects colour perception. It occurs when two different colours seem to be equivalent while there is a difference in the reflected spectrum (Kim-Pusateri et al., 2009, Berns et al., 2002).

Other methods of colour matching include the use of instruments and machines for example spectrophotometers, colorimeters and other systems that involve the use of specific software or digital systems (Chu et al., 2010), refer to Table 1.2 for some examples.

Device	Manufacturer	Type
SpectroShade	MHT Optic Research AG, Niederhasli, Switzerland, Italy	spectrophotometer
ShadeVision	X-Rite America, Inc, Grand Rapids, Mich	digital camera with colorimeter
VITA Easyshade	Vident, Brea, California	spectrophotometer
ShadeScan-	Cynovad, Montreal, Canada	digital camera with colorimeter

Table 1-2 examples of colour measuring devices; table is reproduced from Kim-Pusateri et al., 2009.

Although most colour measuring devices are designed for the same purpose they are different in the way they work, in accuracy and reliability (Kim-Pusateri et al., 2009).
For example, both colorimeters and spectrophotometers employ the CIE system in their analysis of colour. However, they work differently, in that colorimeter require a built in filter to control specimen illumination and light reflection and are much easier to use than spectrophotometers.
However, aging of the filters within a colorimeter results in compromising their ability to measure a colour accurately (Kim-Pusateri et al., 2009, Panariva et al., 2004). Spectrophotometers such as spectroshade are regarded as the most reliable shade matching devices (Della Bona et al., 2009).
The Commission International de l'Eclairage (CIE) set standards for light source and perception in 1931 to represent how a human eye identifies a given colour (Luiz et al., 2007). The biggest advantage of the CIE system is that it enables colour differences to be conveyed in units (Luiz et al., 2007).

1.13.1 The theoretical function of spectrophotometry

Spectrophotometers are devices that can measure the intensity of light passing through an object and are designed in a way that produces standard measures using the CIE system (Igiel et al., 2016), therefore all visible light can be explained and interpreted using spectrophotometers. Measuring colour-using spectrophotometers is regarded as an objective method if compared to direct visual methods with a human eye (Gomez-Polo ey al., 2015).
There are several types of spectrophotometers. There the single beam in which all the passes through the object or the sample as a single beam (Anon, 2017). Other types are double beam where the light is splits into two separate beams one of which passes through a reference point for comparison of intensity. Many devices use a reference point to provide a measurement in relation to the reference point. Therefore the diveces are normally calibrated so whatever measurement is done, is taken in relation to the reference point.
Moreover, information from a colour-measuring device such as the spectrophotometer can be converted into other standard colour measuring methods for example, the vita shade (Igiel et al., 2016).
Results obtained from these devices are improving in terms of accuracy, however, there are some factors that may affect the results in a clinical situation, for example, the contour of a tooth, discolouration of teeth due to aging and loss of translucency (Igiel et al., 2016).

Instruments to measure colour became available in the market during the late 1990s; the first machines were ShadeScan that were developed in Canada (Chu et al., 2010). The main aim has been to increase the effectiveness of shade selection. More machines were developed after that to measure the colour of the entire tooth surface.

1.14 Explanation of the CIE system

The interaction of a light source and a material of a certain colour for example result in light reflection or transmission to the eye. This is affected by the distance between the light source, the material and the eye (Johnston, 2009).
This requires an understanding of three main spectra; the first is the light from the source, the second is the reflected spectrum from an object or material and the third is the spectral observation characteristics of the eye.

The CIE system is the most widely used colour measuring system (Kim-Pusateri et al., 2009) first introduced in 1931 (Luiz et al., 2007); it has been widely involved in the science of dental materials for colour evaluation (Pecho et al.,2016). Using the CIE, colour can be measured in terms of three parameters, brightness or lightness that is normally referred to as (Δ L) and ranges from 0 (black) -100 (white), red-green Chroma (Δ a) and yellow-blue Chroma (Igiel et al., 2016). Therefore, the CIE system is normally written as the CIE L*a*b. This system was introduced and recommended by the CIE in 1976 (Luiz et al., 2007). The other way of expressing the results is by using the CIE Lch system. L is degree of brightness, c is the chroma and h is the hue. Both approaches give the same end scores. Results obtained are normally expressed in terms of mean values of either L*a*b or Lch.
The difference in colour between two measured objects or specimens, for example, is expressed as ΔE. This is an important value that provides a means of quantifying colour (Kim-Pusateri et al., 2009). A ΔE value of 1 or more can be identified or observed by a human eye.

1.15 Colour Stability

The colour of tooth coloured dental materials should ideally not change once placed in the mouth (Mutlu-Sagesen et al., 2005). Discolouration of the material is an unwanted outcome by both the clinician and the patient and may be considered as a sign of failure (Ishikawa-Nagai et al., 2009).
This can be caused by unreacted monomers that are confined in a restoration resulting in an oxidation or degradation process (Sideridou et al., 2012). Additionally,

discoloration or staining of the restoration can be a result of poor marginal integrity and polymerisation shrinkage (Shah et al., 2014). Studies to evaluate colour are either qualitative or quantitative. The latter type of studies can be subjective and can be affected by the limitations of a human eyes perception of colour (Ardu et al., 2011).

1.16 Adhesion of Dental Composite

Adhesion is the process by which the dental material is attached to the tooth surface to improve retention. It is achieved by the twofold mechanism, firstly to enamel and dentine through a micromechanical bond and secondly to the restoration through a chemical bond (Van Landuyt et al., 2007). It is important that a strong link is achieved to the tooth tissues to minimise micro-gaps formation. This ultimately, works in the prevention of failure of the restoration by preventing bacteria gaining access to the interface between the restoration and the tooth (Peutzfeldt, 1997).

Additionally, when materials are bonded to the tooth surfaces micromechanically; the need for removal of tooth tissue is minimised (McCabe and Walls, 2013). Buonocore first introduced the concept of adhesion in 1955; it involves removal or modification of the smear layer, which is left when tooth surface is cut. It is a mixture of debris, tooth tissues and bacteria.

35-37% phosphoric acid is used in order to create a surface free of the smear layer and increase the surface area; on which the adhesive resins will flow and interlocks with the dentinal tubules, thus, creating a hybrid layer. It was by the use of the electron microscopy where Nakabayashi and his colleagues managed to demonstrate the hybrid layer, as the monomer is impregnated into the space created by decalcified dentine (Pashley et al., 2011).

1.17 Adhesive systems

1.17.1 Etch and rinse system

Etching involves removal of the smear layer with the use of 37% phosphoric acid for few seconds to create a demineralised enamel or dentine that is about 5-8 μm (Pashley et al., 2011). The acid etching step is said to improve bond strength (Liaqat et al., 2015). This makes the dentinal surface more permeable, therefore, the resins from the bonding agent can infiltrate. This technique is also known as the three-step technique. This is because it involves etching, priming and bonding using a bonding material. This system has demonstrated a good clinical out come and has lower failure rates compared to other adhesive systems and is regarded as the gold standard (Moretto et al., 2013). The main disadvantages of the three-step system are that it is

time consuming and the relatively large hybrid layer i.e. the maybe at risk of more degradation on exposure to water. Additionally, more hydroxyapatite is removed with this system, making the possibility of a chemical reaction between the resin and hydroxyapatite ultimately low (Moretto et al., 2013).

1.17.2 Self-etch system

This is also known as the two-step system and it basically involves modification of the smear layer. In a recent randomised clinical trial: this system showed a superior clinical performance (Peumans et al., 2015). Some studies indicated that the two steps system had less annual failure rate when compared to the three-steps system (Moretto et al., 2013). Although the primary bonding mechanism is through the micromechanical means; the smear layer is not totally removed and so the likelihood of a chemical bond is higher, therefore bond strength is improved. This is also thought to reduce post-operative sensitivity. Additionally, fewer steps are involved with self-etch systems.

1.17.3 One-step adhesion (all in one)

This is an adhesion system that combines the three steps (etching, priming and bonding) in a single step making the bonding process easier and faster. Examples of materials using these systems are EasyBond and TotalBond both contain 4-META (Jeffrey Chai Chang et al., 2002). The main concern with this system is that high solvent concentration is used in order to keep both the hydrophilic and hydrophobic monomers in the system. As studies have shown, this is more likely to produce nanoleakage (Torkabadi et al., 2008). Additionally, the hydrophilic monomers are highly susceptible to water absorption. This may result in compromising the bond and failure of the material.

1.18 Current trends in dental composite materials

In modern caries management techniques, clinicians are more reluctant to remove deep dentine that is likely to remineralise (Mehdawi et al., 2009) and the general trend is directed towards minimal invasive dentistry (Cocco et al., 2015). However, concerns have been raised because of the possibility of recurrent caries when infected dentine is left behind. Thus, current considerations in dental material science are directed towards the incorporation of antibacterial materials particularly within dental composites. This can be done by modification of contents either in the liquid or the powder phase of dental materials (Liu et al., 2013, Imazato,2003).

This is also because dental composite restorations are more susceptible to biofilm

accumulation than other restorative materials (Jingwei Hea, 2014).

A recent systematic review concluded that it is beneficial to incorporate antibacterial monomers into an adhesive system (Cocco et al., 2015) because this will minimise the possibility of biofilm accumulation.

The main antibacterial agent used previously is Fluoride, integrated in lining materials such as glass ionomers. Besides its ability to form hydroxyfluoroapatite; the antibacterial effect here is mainly through the incorporation of Fluoride into the tooth structure. Fluoride is also known for its inhibitory effect on plaque bacteria.

Fluoro-apatite is much stronger and resistant to acidic attack than hydroxyapatite. A very high pH of a material produces some anti-bacterial effects, as the case with Calcium hydroxide based materials. A main problem with these materials is that they normally dissolve into body fluids and do not adhere to the composite materials (Mehdawi et al., 2009). Chlorhexidine has also been one of the main ones to be used due to its ability to destroy bacteria.

Additionally, beside the incorporation of antibacterial and remineralising agents, the modern concept is to produce materials that are self-adhesive by including acidic monomers such as 4META (Pinna et al., 2015).

1.19 Antibacterial agents used in dental composite systems

1.19.1 Chlorhexidine

Chlorhexidine digluconate is a well known antimicrobial agent that has been used for many years. It is active against Gram positive and Gram-negative bacteria as well as fungi, yeast and some viruses. It has a molecular weight of 505.4 g/mol. It is a dicationic molecule and its actions are mainly through the attachment of it is two ions to the bacteria membrane on one side and the dental pellicle on the other side.

Many oral care products contain chlorhexidine as a means of caries and periodontal disease prevention. These are normally in the form of: toothpastes, mouthwashes, varnishes, gels, and sprays. Chlorhexidine is regarded as a broad-spectrum antimicrobial agent. It is both, bactericidal i.e. results in death of the bacteria and a bacteriostatic i.e. prevents growth of the microbes when used in moderate concentrations. Commonly used concentrations are 0.2% and 0.1% as in mouthwashes and rinses or 1% as in dental gels.

Side effects of chlorhexidine include, a bitter metallic taste that is felt for some time after the use of chlorhexidine mouthwash and commonly staining of teeth and tongue temporarily. Mucosal irritation and Life threatening hypersensitivity with chlorhexidine has also been reported (Walsh et al., 2015).

1.20 Quarternary Ammonium (QA) salts

QA is a positively charged well known antibacterial agent that has a low toxicity and a broad spectrum antibacterial action (Jingwei Hea et al., 2014). Quartenary ammonium methacrylate monomers can bind to other monomers within a composite system and a long-term anti-bacterial action may be possible (Cocco et al., 2015). The antibacterial effect of QA is through its ability to diffuse within the cell membrane of the bacteria and distraction of its cytoplasmic membrane. Methacryloyloxydodecylpridinium bromide MDPB is derived from QA and is the first QA to be used in dental materials as an antibacterial agent (Cocco et al., 2015).

1.20.1 Benzalkonium chloride (BAC)

BAC is an antimicrobial product has been included in acid etchant products by some manufacturers for many years such as the Bisco (Schaumburg, USA) (Tezvergil-Mutluay et al., 2011). It has been available as an antimicrobial agent since 1935 and has a wide safety margin (Tezvergil-Mutluay et al., 2011, Marple et al., 2004). BAC is composed of a number of different alkylbenzyl-dimethylammonium chlorides; it contains a nitrogenous cation that contains a quaternary ammonium group (Tezvergil-Mutluay et al., 2011, Marple et al., 2004). BAC is also used in chemistry as a transmission agent from on phase to another (Tezvergil-Mutluay et al., 2011).

1.21 12-methyacryloyloxyldodecyl-pyrimidium bromide (MDPB)

MDPB is a monomer that is normally incorporated in dentinal adhesive agents and kills bacteria by bactericidal action (Tziafas et al., 2007). The antibacterial effect is caused by MDPB binding to the negatively charged bacterial cell membrane (Imazato et al., 2003).

1.22 Silver particles

Silver particles have been considered as antibacterial agent in small percentages 0.1-1%. This is because silver has antimicrobial effect and is very biocompatible. The antibacterial action is through interference with the formation of bacterial DNA (Cocco et al., 2015). However the incorporation of silver in dental composites has been linked to instability of colour (Imazato, 2003). The use of nano-sized silver particles has been advocated in several fields including the medical field (Sarsar et al., 2014) due to its wide range antimicrobial activity. Silver particles are effective against several pathogenic organisms such as, *Staphylococus aureus* and *Escherichia coli* (E.coli) (Sarsar et al, 2014).

1.23 E-Polylysine (Polylysine)

Polylysine is naturally occurring pale yellow material that is produced from fermentation of aerobic bacteria by a non-pathogenic microorganism known as *Streptomyces albulus* (Shih et al., 2006, Hiraki et al., 2003), which is a gram positive bacteria that was isolated from Japanese soil. The molecular weight of e-polylysine produced via this route is about 4700 and the lysine molecules are linked by the epsilon. E-polylysine molecules are cationic with positively charged amine groups in water (Hiraki et al., 2003).

Polylysine is a homo-polypeptide molecule that has residues of L-lysine estimated as 25-30 (Yoshida and Nagasawa, 2003, Hiraki et al., 2003). A peptide bond links the carboxyl and the e-amino group.

Polylysine has been recognized by the American food and drug administration as safe for use in food up to levels as much as 50 mg/Kg (Ye et al., 2013). Disintegration of polylysine results in the formation of lysine molecules without any side effects to the cells. Several papers reported that even when taken in high doses no carcinogenic affects were noticed (Neda et al., 1999, Hiraki et al., 2003 and Ye et al., 2013).

Polylysine, has very high water solubility and is biodegradable (Shih et al., 2006). Its use has been advocated in several fields, including food industry, medicine and electronics. The antimicrobial properties it is used as widely as a preservative agent (Shih et al., 2006). Additionally, it is unscented and tasteless making it an ideal preservative. In Japan, it is widely used as a preservative for food such as meat, sushi and rice, in relatively low concentrations (Ye et al., 2013).

Polylysine is known to have broad-spectrum antimicrobial properties. It can impede the growth of a wide range of microbes including yeast, bacteria both gram positive and gram negative(Yoshida and Nagasawa, 2003).

The antimicrobial action is believed to be through adsorption into the cell membrane, resulting in abnormalities in the cytoplasm (Najjar et al., 2009).

There are different ways by which polylysine release can be assessed. One of the methods is by using the UV-spectrometry. In which solutions obtained from immersion of polylysine containing composite discs are placed into the machine to obtain the absorbance of the solutions with Trypan blue assay method.

The second method is by using HPLC (High performance liquid chromatography), which involves using a specific machine where the storage solutions are placed into specific vials and measured using HPLC. Both methods involve the use of standard polylysine solutions for calibration. In this research experemint HPLC was used to determine the release of polylysine.

Standard polylysine solutions were prepared in order to generate a calibration curve by dissolving polylysine in distilled water.
Other techniques of measuring polylysine release were used in previous projects at EDI, is by using UV spectrometer, which is described in the techniques chapter.

1.24 Triclosan

Triclosan is widely used antimicrobial substance that is regarded as a broad spectrum (Kalyon et al., 2001). It has been used in industrial products such as deodorants for decades (Wicht et al., 2005). It is particularly effective against Gram positive bacteria (Rathke et al., 2010) making it widely being used in dental care products. The main action is by acting on bacterial enzymes and inhibiting growth. Triclosan has been recently banned by the food and drug administration (FDA) to be used in soap products (McNamara et al., 2016) due to fears of it being involved in antibiotic resistance.

1.25 Remineralising agents used in dental composites

1.25.1 Fluoride

Fluoride is a naturally occurring element. Fluoride from toothpastes and other sources is adsorbed to the tooth structure and results in the formation of the much stronger acid resistance Fluoro-apatite.

However, fluoride has also been associated with many health issues, including cancer. Although, none of the claims that fluoride can cause cancer are as yet proven, fluoride has been confirmed to cause dental and bone fluorosis when taken in high concentrations.

1.25.2 Calcium phosphate (Ca PO_4) and its derivatives

In a human body calcium and phosphate are the most important elements forming the hard tissues namely bone, teeth and cartilaginous tissues to provide support (Dorozhkin et al., 2002). In the world of biomedical studies, calcium and phosphate are the two minerals that have drown the interest of scientists as replacements of teeth and bone (Sanchez-Enriquez et al., 2013).

There are several types or phases of calcium phosphate materials widely used in deferent fields of medicine and industry. This is because calcium phosphate materials are largely biocompatible.

In its different forms, calcium phosphate has been greatly used as a coating mineral for other minerals used in medicine for example (Shadanbaz et al., 2012).

Calcium phosphate can occur in several types depending on the amount and ratios of both the calcium and phosphate ions. Examples include, amorphous calcium phosphate (ACP), dicalcium phosphate anhydrous (DCPA), dicalcium phosphate dehydrate (DCPD) also known as brushite, monocalcium phosphate anhydrate (MCPA) and monocalcium phosphate monohydrate (MCPM) and several others as demonstrated in (Figure 1.5).

The ratio of calcium to phosphate in the different compounds affects their solubility.

The incorporation of calcium-Phosphate materials may have a lot of benefits, in that they provide Calcium and phosphate ions that may contribute in remineralisation of an infected tooth surface. Sonication is a process by which different phases of calcium phosphate can be produced (Sanchez-Enriquez et al., 2013).

1.25.3 Production and creation of calcium phosphate

Calcium phosphate can be produced using hydrothermal methods where materials such as hydroxyapatite and CaO with P_2O_5 are combined under high temperature and pressure (Hsu et al., 1998, Sanchez-Enriquez et al., 2013).
Microwave systems have also been used in some studies (Kong et al., 2005) by mixing emulsion mixtures of calcium and phosphate under controlled conditions. Other reported methods include the use of ultrasound devices to produce different forms of calcium phosphate (Sanchez-Enriquez et al., 2013).

1.25.4 Amorphous calcium phosphate (ACP)

ACP has been found in the eukaryote and prokaryote mitochondria (Combes and Ray, 2010). The term is used describe an amorphous form of calcium phosphate, or poorly organised apatite structures either of organic or synthetic origin as well as molecular aggregations of cheese and milk products (Combes and Ray, 2010).
It was first incorporated in dental composites during the 1990s (Skrtic et al., 2004).
In a study conducted recently the author stated that main problem identified with some ($Ca\ PO_4$) composite materials was the inability to control the aggregation, particularly with aqueous Calcium Phosphate materials ACP; this resulted in inferior mechanical properties of the composite material (Antonucci et al., 2009).

1.25.5 Monocalcium calcium phosphate monohydrate (MCPM)

MCPM has a lower calcium phosphate ratio than ACP 0.5 compared to 1.2-2.2 (Dorozhkin et al., 2002), and has the highest solubility in water compared to the other calcium phosphate compounds (Dorozhkin et al., 2002).
It is used in industry as a buffering and a hardening material, also as in food products as yeast (Sanchez-Enriquez et al., 2013).
MCPM is acidic and the interaction of MCPM and water results in the formation of phosphoric acid and either dicalcium phosphate dihydrate (DCPD) (brushite) oranhydrate (DCPA) (monetite) due to hydrogen ion movement. It has been used in several researches at the Eastman dental Institute (EDI) as a component that can promote re-mineralisation as well as the promotion of water diffusion into the material to compensate for shrinkage (Liaqat, 2015).

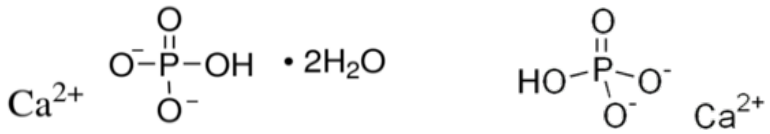
1.25.6 Mineral Brushite (DCPD) and Monetite (DCPA)

Brushite was discovered in 1989 by Mirtchi and Lemaitre and resulted from MCPM interactions (Tamimi at el., 2012). Widely used in medicine and dentistry to replace the missing hard tissues, mineral brushite or dicalcium phosphate dihydrate (DCPD) has become the focus of several studies as it can be used for the formation of hydroxyapatite as well as synthetic bone cements (Shadanbaz et al., 2012). Additionally, it is inexpensive to manufacture (Shadanbaz et al., 2012) and can be formed by a crystallisation process in acidic aqueous solutions.

When a phosphoric acid (H_3PO_4) solution is mixed with calcium hydroxide suspensions $Ca(OH)_2$ in equal amounts of moles at room temperature, Brushite (DCPD) will be formed (Oliveira et al., 2007).

Monetite can be formed from brushite when temperatures about 80°C are used during the procedure. Removing water molecules from the brushite forms Monetite, it is also referred to as the anhydrous form of DCPD (Sanchez-Enriquez et al., 2013).

Both DCPD and DCPA have been used variously as calcium-phosphate cements sources of calcium and phosphate ions and as toothpaste ingredients.



(1) (2)

Figure 1.4 the chemical structures of Brushite (1) and Monetite (2)

1.25.7 Hydroxyapatite

Hydroxyapatite is the main component of the tooth and bone structures (Sadat-Shojai et al., 2013). The crystal unit of hydroxyapatite is normally comprised of two molecules, therefore the chemical formulation is normally written as $Ca_{10}(PO_4)_6(OH)_2$ instead of the single unit representation $Ca_5(PO_4)_3(OH)$.

Both hydroxyapatite and the fluoride-containing version of the apatite (fluoroapatite) are very stable in terms of the chemical structure.

The main two elements in the hydroxyapatite are Calcium and phosphate, with a ratio of 1.67 (Hench et al., 1993) and this is regarded as ideal for dental tissue remineralisation. This is because a ratio above 1.67 it is regarded a calcium rich hydroxyapatite. On the other hand, a ratio, which is below 1.67, indicates a calcium

deficient hydroxyapatite that contains some impurities or weaker phases (Hench et al., 1993).

1.25.8 Synthetic hydroxyapatite

Many studies have been conducted regarding developing a synthetic form of hydroxyapatite (Sadat-Shojai et al., 2013) and many techniques have been employed. These can be divided into dry methods such as the solid-state synthesis or wet methods such as hydrothermal, precipitation and hydrolysis.

Dry methods are generally cost effective and require fewer chemicals mixed under very high temperatures up to 1000 ^{o}C.

Physiognomies of hydroxyapatite depend on several factors, for example, the way the crystals are arranged, their morphological features, stoichiometric features and the presence of other phases including those with impurities (Sadat-Shojai et al., 2013). These chemicals are usually salts of calcium and phosphate. The main disadvantage of dry methods is that the resultant hydroxyapatite normally comprises of big and irregulary shaped crystals Sadat-Shojai et al., 2013).

Wet methods of hydroxyapatite preparation, have the advantage that the size and morphological features of hydroxyapatite crystals can be controlled.

This method is of variable cost but is regarded easier as stated at the (Sadat-Shojai et al., 2013).

Chemical precipitation method is regarded as the easiest and most conventional way of producing hydroxyapatite (Kim et al., 2009).

Ca/P ratio	Compound	Formula	Solubility at 25 °C, −log(K_{sp})	Solubility at 37 °C, −log(K_{sp})	pH stability range in aqueous solution at 25 °C
0.5	monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2 \cdot H_2O$	1.14	no data	0.0–2.0
0.5	monocalcium phosphate anhydrate (MCPA)	$Ca(H_2PO_4)_2$	1.14	no data	[d]
1.0	dicalcium phosphate dihydrate (DCPD, "brushite")	$CaHPO_4 \cdot 2\,H_2O$	6.59	6.63	2.0–6.0
1.0	dicalcium phosphate anhydrate (DCPA, "monetite")	$CaHPO_4$	6.90	7.02	[d]
1.33	octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4 \cdot 5\,H_2O$	96.6	95.9	5.5–7.0
1.5	α-tricalcium phosphate (α-TCP)	$\alpha\text{-}Ca_3(PO_4)_2$	25.5	25.5	[b]
1.5	β-tricalcium phosphate (β-TCP)	$\beta\text{-}Ca_3(PO_4)_2$	28.9	29.5	[b]
1.2–2.2	amorphous calcium phosphate (ACP)	$Ca_x(PO_4)_y \cdot n\ H_2O$	[c]	[c]	[e]
1.5–1.67	calcium-deficient hydroxyapatite (CDHA)	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $(0< x <1)$	≈85.1	≈85.1	6.5–9.5
1.67	hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	116.8	117.2	9.5–12
2.0	tetracalcium phosphate (TTCP)	$Ca_4(PO_4)_2O$	38–44	37–42	[b]

Figure 1.5 The different types of calcium phosphate (Dorozhkin et al., 2002).

1.26 The need for a new material

The demands for replacing dental amalgam are increasing since the Minamata convention on mercury in 2013. The urge for developing new materials, which are biocompatible, is growing.

Resin composites are the best materials in terms of mechanical properties after amalgam. However, there are still many drawbacks associated with composites as discussed in detail previously. Therefore, there is a need for improving resin composite materials.

Adding to that, composite materials require several steps for placement and controlled working conditions. This makes them time consuming and difficult to place. Several studies indicated the impact of difficulty of using dental composites in a general practice compared to the other commonly used material (dental amalgam) (Naghipur et al., 2016).

The current study focuses on developing a novel dental composite material with a particular focus on improving the monomer phase, by creating a monomer solution that is clear of solid particles as this may improve biocompatibility.

In addition, PPGDMA was used as the diluent monomer for the final material as it has been shown to improve monomer conversion in previous EDI projects.

Moreover, the project focuses on improving and promoting other desirable features in composite dental materials such as promoting remineralising and antibacterial properties by using materials e.g. MCPM as a remineralising agent and antibacterial e.g. PLS. Water sorption colour stability were also assessed.

1.27 Aims and objectives

1.27.1 The aim

The focus of this research is on developing a new composite material, with good setting characteristics, dimensional / colour stability in addition to the incorporation of antibacterial agents such as polylysine, and the addition of components with potential to enable tooth remineralisation such as MCPM.

Other features such as adhesion and remineralising action, effect on mechanical properties such as biaxial flexure, wear and bonding strength are covered in other previously undertaken at the EDI.

The main aim of this study is to optimise the properties of the colour and monomer conversion of novel biocompatible and antibacterial dental composites.

This project examines:

- The effects of omitting the amine DMAEMA in monomers containing 1 wt% CQ.
- The maximum amount of 4META that can be used if DMEAM is omitted.
- Can maximum monomer conversion be achieved in composites containing PPGDMA.
- If Maximum water sorption can be achieved in formulations with different concentrations of MCPM and PLS.
- If maximum PLS release in composites containing 2 or 5 wt% PLS is affected by the powder liquid ratio (PLR).
- Colour stability of novel composites of different PLR and containing PLS and MCPM after immersion in commonly used solutions.

1.27.2 Objectives:

This project examined the monomer conversion, the colour and clarity properties, the colour stability in different solutions, the expansion properties, and the potential anti-bacterial release of the polylysine in the following experiments:

1. UV spectrometry of monomers with different % of CQ, and the effect of DMAEMA addition on clarity of the different monomers
2. Using UV spectrometry to assess the effect of lowering the amount of 4META on clarity of the monomer.
3. Monomer conversion using FTIR
4. Involvement of MCPM to promote the water sorption potential by quantifying the mass, density, and volume change during 8 weeks in de-ionised water.

5. Observational colour assessment of the effect of immersing composite discs in different solutions.
6. Quantifying the degree of colour change when discs of the novel composites immersed in three solutions using the spectroshade and comparing the effect with a commercial material.

Chapter Two

Materials & methods

2 Materials & methods

2.1 UV spectroscopy

UV spectrometers can be used to measure how much light a material absorbs. When a beam of light passes through a solution, photons from the light interact with the molecules in the solution. Some of the photons are absorbed and others are reflected or scattered. Visible light ranges from 400-700 nm while the ultraviolet light range is from 10-400 nm.

2.2 UV spectrometers

UV spectrometers produce light that passes first through a collimator or a prism, splitting the light into a range of wavelengths. These then pass through a wavelength selector, and then through the reference and the sample solutions to the detector. The initial beam has intensity I_0 and the detected beam intensity is I.

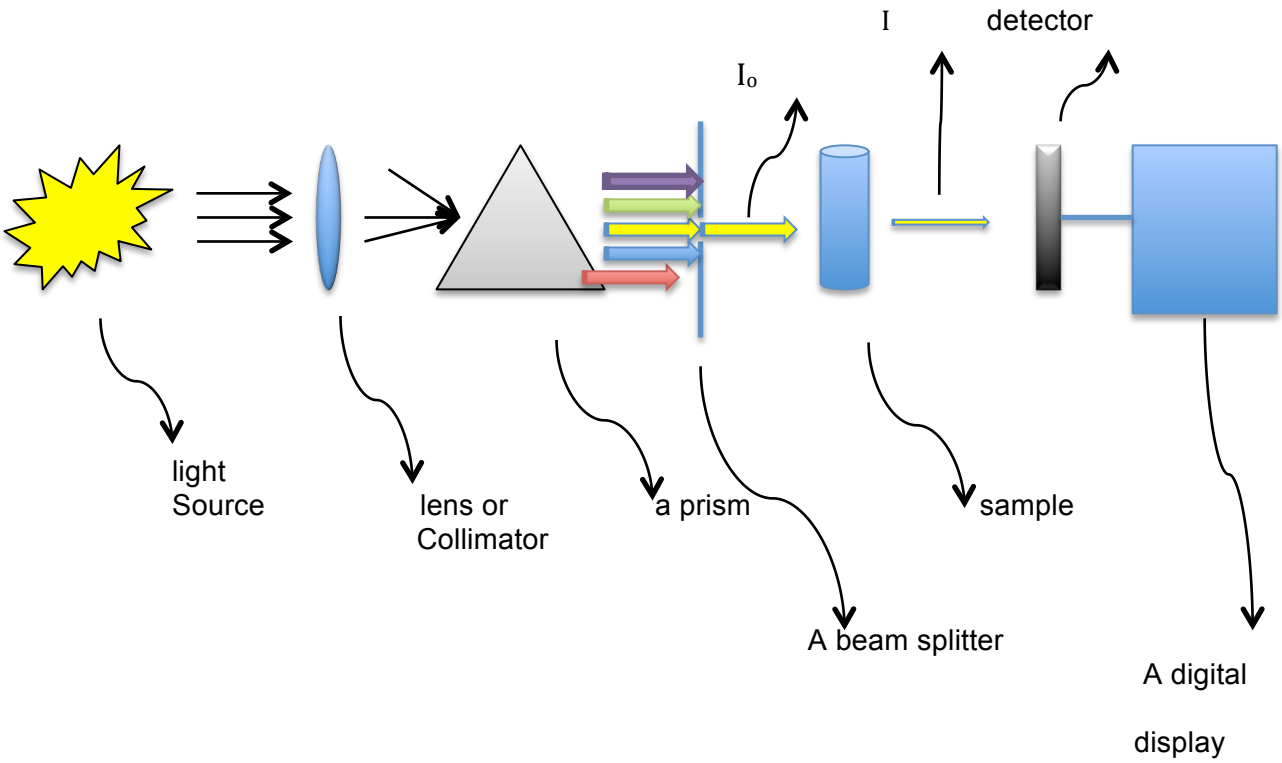


Figure 2.1 A basic diagram of UV-visible light spectrometer.

Beer Lambert law

According to the Beer-Lambert law, absorbance of a solution at a given wavelength is proportional to concentration and path length. The more concentrated a solution is the more molecules will be available to interact with light, and the higher the absorbance. The equation is as below:

$$A = \log_{10} \frac{I_\circ}{I} = \mathcal{E}lc \qquad \text{Equation 1}$$

$I_\circ$ = light intensity before it passes into a sample

I = light intensity after it passes the sample

$\mathcal{E}$ = Absorption coefficient

l = path length

c = concentration

Prepared monomers were placed in cuvettes that had a path length of one centimeter. A Unicam Ultraviolet-visible (UV) 500 spectrometer (Thermo Spectrotonic, UK) was employed. Absorbance was obtained between 400 and 600 nm.

2.3 Calculating reaction rates

Reaction rates were calculated from the results obtained from the FTIR, by calculating the maximum conversion divided by time in seconds.

2.4 Measuring monomer conversion (MC) using infrared spectrometry

Infrared wavelengths range from 700 to 1050 nm (Lynch and Livingston, 2001). Using IR it is possible to study the molecular structure of materials and energy changes associated with molecular vibrations. When a molecule absorbs an infrared photon it gains energy and molecular bonds are excited to higher vibrational energy levels (Stuart, 2005).

2.4.1 The concept of FTIR (Fourier Transform Infrared)

Fourier Transform Infrared (FTIR) can be used to determine monomer conversion (Halvorson et al., 2002) and has been used extensively to determine conversion of methacrylate monomers in dental composites (Ho and Young, 2006). The FTIR concept is mainly based on Albert A. Michelson theories (Newport.com, 2015,

accessed March/2015). Measuring infrared absorbance can provide information regarding molecular structure. The infrared light can be divided into three levels according to the wavelength or wavenumber. Far infrared has a wavelength range of 200-10 /cm; mid infrared ranges between 200-4000 /cm and near infrared is 4000-12800 /cm.

Real time monitoring of the polymerisation progress is possible with the use of attenuated total reflection (ATR) FTIR. In this thesis, an ATR FTIR (Perkin-Elmer, Series 2000 UK) was used to determine monomer conversion of the composites at normal body temperature of 37°C. The background spectrum was first scanned before starting the experiment. Samples were scanned for 15 minutes and data analysed using Timebase software. The spectra obtained had a resolution of 8 cm^{-1} and a range from 700 cm^{-1} to 2000 cm^{-1}. For each composite scanning was repeated at least three times for accuracy (n=3).

Monomer conversion was calculated using the formula below:

% Of Monomer conversion (MC) $= 100\left(1 - \frac{A_t}{A_0}\right)$ **Equation 2**

A_o and A_t are peak absorbance at 1320 cm^{-1} above background at 1334 cm^{-1} before and after curing. This corresponds to the CO stretch in the methacrylate, which shifts to lower wavenumbers upon polymerization.

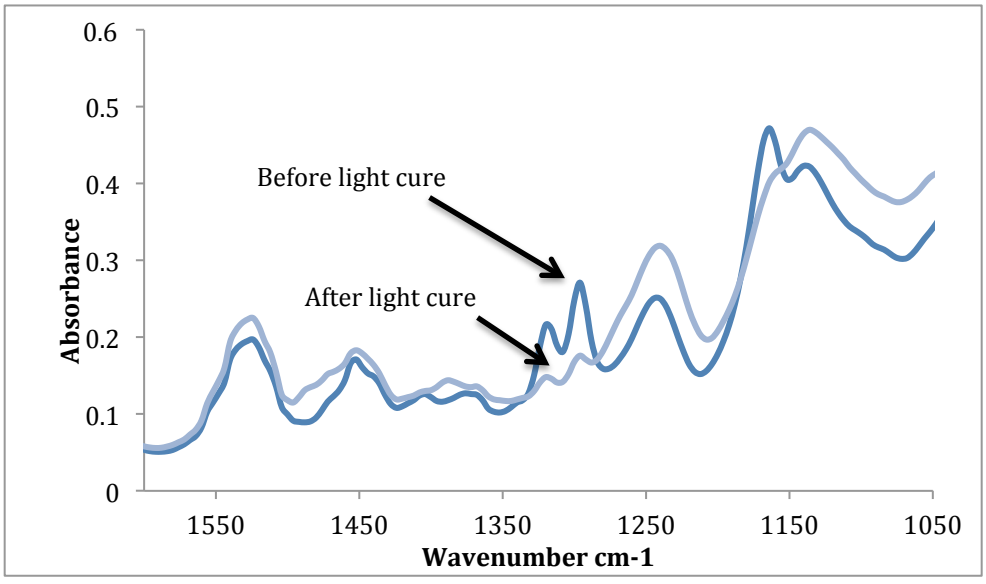


Figure 2.2 an example of a spectrum of monomer before and after curing

2.5 Mass and volume change in water, for composite materials containing different percentages of MCPM and PLS

To assess mass and volume change set composite discs of 1mm depth and 1 cm diameter were stored in 10ml of water in sterilin tubes at 37˚ C in an incubator. The mass was obtained in air and water, after 0, 1,3 and 6 hours, then 3 and 7 days then 2,3,4,5,6,7 and 8 weeks using a four-figure balance (OHAUS Pioneer Series) and density tool kit. Dry weight for each sample was checked twice and three times in water and the average was calculated. Temperature was recorded. The following equations were used (taken from the density toolkit manual) (Mettler Toledo, Leicester, UK).

$$\rho = \alpha \frac{A-B}{V} + \rho_L \qquad \text{Equation 3}$$

$$\rho = \alpha \frac{P}{V} + \rho_L \qquad \text{Equation 4}$$

A = Weight of the sinker in air

B = Weight of the sample in the liquid

V = Volume of the sinker

ρ_L= Density of air (0.0012 g/cm3)

α = Weight correction factor (0.99985), to take the atmospheric buoyancy of the adjustment weight into account

P = Weight of the displaced liquid (P = A-B)

Averages and standard deviations for three samples were calculated and plotted versus the square root (SQRT) of time.

2.6 High performance liquid chromatography

High performance liquid chromatography (HPLC) was used to determine polylysine release. (In this thesis data of Nabih Alkhouri are presented due to multiple problems with equipment breakdown limiting access to the technique). This technique separates and then quantifies components in solution via UV absorbance. In the study reported in this thesis the storage solutions used during mass and volume change investigations are used to assess polylysine release.

HPLC is used in fields such as the medical field, industrial and in chemistry. It has been used to quantify the amount of monomers such as HEMA (Nicholson and Czarencka, 2008) that are regarded as toxic if leached out from the filling material. The

use of HPLC for determination of monomer conversion has also been reported in literature (Shin and Rawls, 2009). To check the amount of cumulative PLS released over time solutions obtained after immersion of composite discs are placed into 0.3 ml vials which can fit into the automatic sample changer of the HPLC instrument. The instrument uses a pump system that drives high pressure liquid into a column filled with adsorbent. UV absorbance peaks for the PLS are obtained and compared with results generated from PLS solutions of known concentrations.

2.7 Spectroshade colour evaluation instrument

The spectroshade micro spectrophotometer (Version 2.4) system with a removable and sterilisable mouthpiece is produced by Medical High Technology (MHT) by (Enrico Fermi 22, 37135 Verona (VR)) Italy. To determine the shade of a filling or a tooth an image of the tooth is taken by the spectroshade camera.

A mouth simulator box (supplied by Medical High Technology (MHT)) is used to arrange the composite discs and simulate the clinical situation. The mouth simulator box also blox any external light. Pink plasticine was used to simulate the gingivae and to support the composites discs (Figure 2.3).

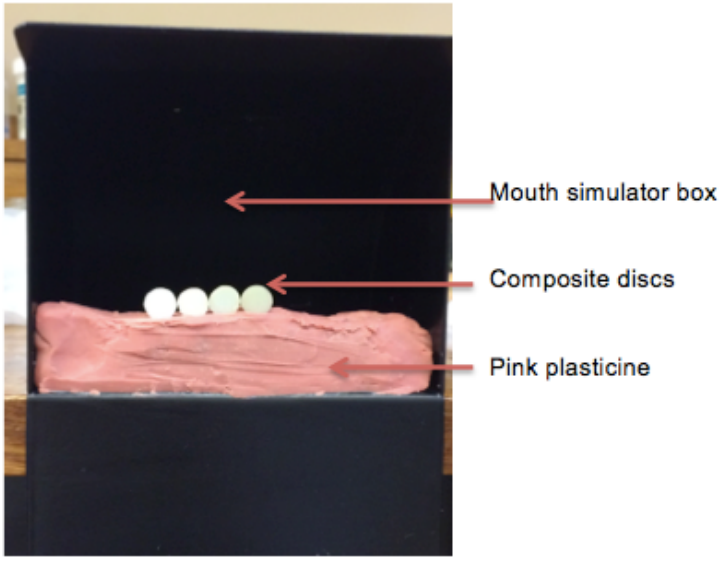


Figure 2.3 Mouth simulator box and pink plasticine supplied by (MTH, Italy)

2.8 Spectroshade colour evaluation method

The spectroshade device is calibrated using white and green calibration tiles supplied by the manufacturer and located on the docking station; this has to be done each time before an image is taken. The machine was held directly against the composite disc

until a clear image was displayed on the screen by clicking on the Start Live on the screen.

The surrounding light does not affect the readings provided by the machine, as the mouth piece blocks external light. Once the image is detected angulation lines appear on the disc and an image is taken.

Images can then be transferred into a computer using spectroshade downloader application after installing the software using a CD provided by the manufacturer.

Form the display screen on the spectroshade device; the user can select the way an image taken by the machine and the shade.

This can be done by selecting an option from the toolbar, for example, an over all shade of the tooth or composite disc can be given, or the displayed image can be divided into several sections or zones and a shade value is given for each zone separately as shown on (Figure 2.4).

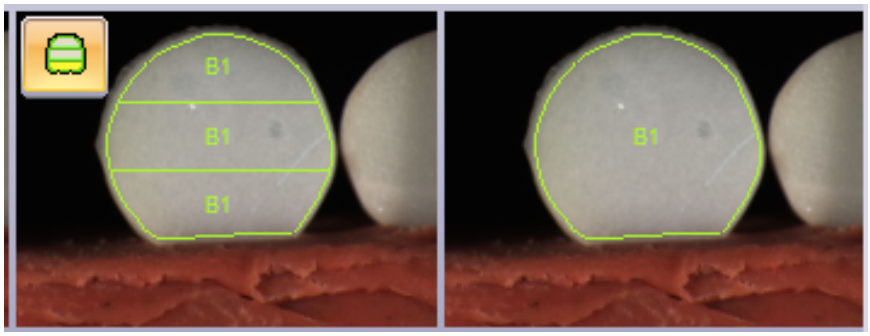


Figure 2.4 an example of how spectroshade was used in this project. Composite shades as displayed on the screen.

Using the toolbar on the spectroshade screen, images can be compared and analysed either by using the CIE L*a*b or CIE Lch as below (Figure 2.5).

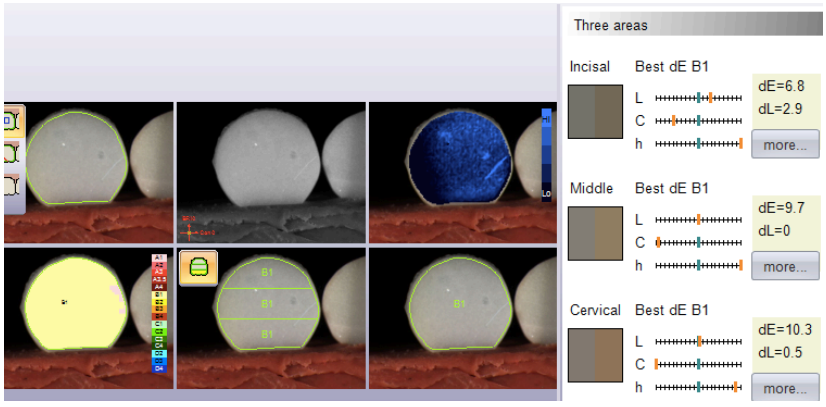


Figure 2.5 examples on how composite discs are analysed and compared with other images on spectroshade.

Specific point analysis of the colour can also be done using the spectroshade software, as demonstrated in (Figure 2.6). In this experiment, in order to minimise bias, a reference point was selected from a disc that is not immersed from the middle of the disc. This was compared to three randomly selected points, one in the middle, one from the top of the composite disc and one from the bottom of the disc. All three points were compared to the reference point in terms of CIE Lch. Colour change values ΔE were then obtained. Results were then expressed in terms of ΔE, three readings were taken for each samples and standard deviations were calculated.

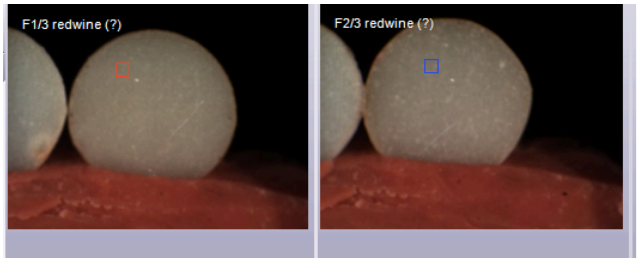


Figure 2.6 Point analysis using spectroshade to check if the colour is variable at different points

2.9 Materials

Several monomers were used in this project for the preparation of composites. These were combined with varying levels of initiators and activators. A list of monomer components is provided in Table 2-1 along with batch numbers, suppliers and chemical formulae.

Name	Abbreviation	Formulation	Supplier	Batch No & date	Function
Urethane dimethacrylate	UDMA	$C_{23}H_{38}N_2O_8$	DMG, Germany	July/2014 # 90761	Bulk monomer
Poly(propylene glycol) dimethacrylate	PPGDMA	$H_2C=(CH_3)C$ O $(OC_3H_6)_n$ O_2C C $(CH_3)=CH_2$	Polysciences , Inc.	July/2014 # 04380	Diluent monomer
Triethylene-Glycol Dimethacrylate	TEGDMA	$C_{14}H_{22}O_5$	Polysciences , Inc.	July/2014 # 16589	Diluent monomer
4- Methacryloy loxy ethyl trimellitate anhydride	4META	$C_{15}H_{12}O_7$	Polysciences , Inc.	July/2014 # 17285	Adhesion
Camphorquino ne	CQ	$C_{10}H_{14}O_2$	DMG, Germany	July/2014 # 90339	Photo-initiator
2-Dimethyl-amino ethyl methacrylate	DMAEMA	$C_8H_{15}O_2N$	DMG, Germany	July/2014 # 04467	Tertiary-amine & activator

Table 2-1 monomers used in the experiments, with abbreviations, chemical formulae, supplier details, batch numbers and dates of supply and their functions.

Suppliers, batch numbers and functions of composite filler phases are summarised in Table 2.2. SEM images of the particles used are given in Figures 3.1-3.4. The filler phase for the basic composite consisted only of glass particles. This had an average diameter of 7μm (DMG, Germany) (Figure 2.7). The PLR for basic composites was 4. Active composites contained "hybrid" glass of 7 micron: 0.7 micron: nano silica 6:3:1 by weight to enable maximum packing of the filler. PLS particles (Figure 2.9) were used

as an antimicrobial agent, and MCPM particles (Figure 3.4) as a remineralising material. For active composites containing PLS and MCPM, PLR was 3,4 or 5.

Name	Abbreviation	Supplier	Batch No/code & date	Function
DMG glass Norm.Sil	DMG 7µm	DMG, Germany	July/2014 # 680326	Improving the mechanical properties
DMG glass	DMG 0.7µm	DMG, Germany	March/2014 # 706366	To fill spaces between larger particles
Nano glass	Nano glass	Evonik Industries AG, Germany	August/2013 # 90761	To aid flowability and further enhance packing density
Monocalcium phosphate monohydrate	MCPM	Himed	July/2014 # MCP-369925	Remineralising agents
Polylysine	PLS	Handary, Belgium	March/2016 #	Antibacterial agent

Table 2-2 Components of the various fillers used with abbreviations, batch numbers and functions

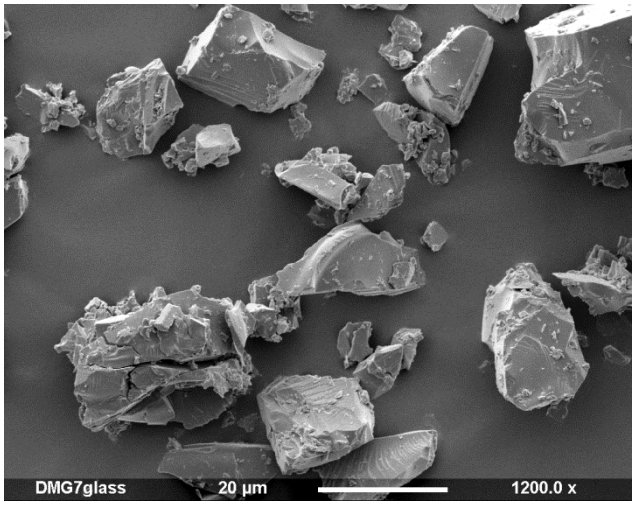


Figure 2.7 SEM image of DMG 7 µm glass (courtesy of Dr Wendy Xia)

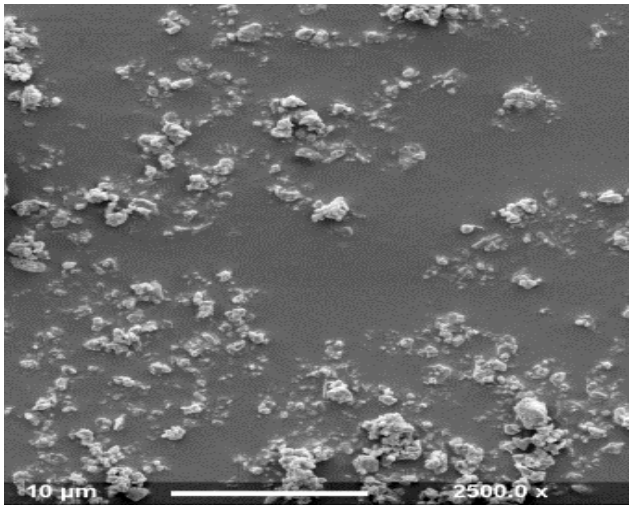


Figure 2.8 SEM image of DMG 0.7 µm glass (courtesy of Dr Wendy Xia)

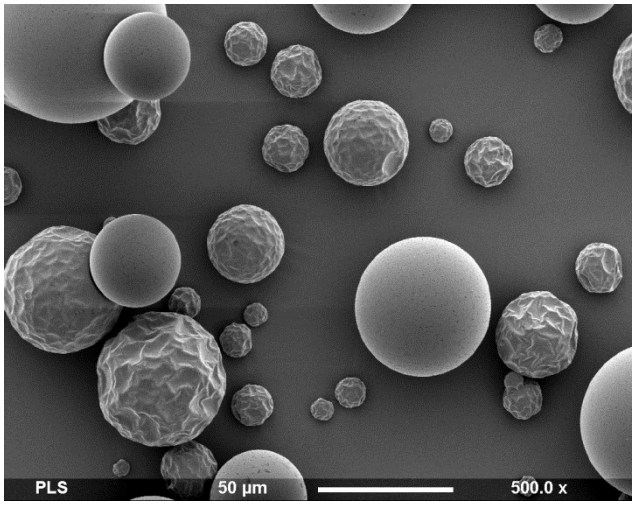


Figure 2.9 SEM image of polylysine particles (courtesy of Dr Wendy Xia)

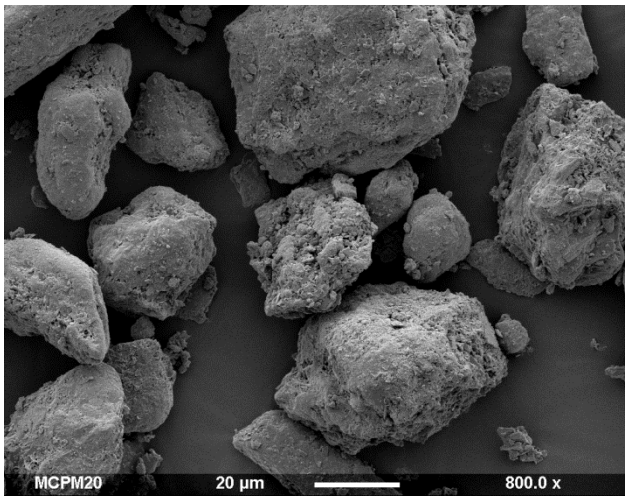


Figure 2.10 SEM image of MCPM (courtesy of Dr Wendy Xia)

2.10 Preparation of monomers and assessment of their colour and clarity

For composite preparation, it is important to produce clear liquid phases to ensure reproducibility. Monomers that are not fully dissolved may not fully polymerised and ultimately leach out of the material, undermining biocompatibility. Furthermore, they may sediment with time thereby affecting shelf life. Initial work suggested sample cloudiness could be improved by adding DMAEMA (wt%) probably due to an interaction with 4META. Additionally, an optimised level of CQ is important to ensure effective polymerization. A number of monomers were therefore prepared with varying levels of these components and different diluent monomers.

Undissolved particles will cause scattering affecting clarity and causing background scattering in UV spectra. Light needs to be absorbed by the initiator to start the polymerization process. UV spectrometry was therefore used to determine the absorbance and scattering due to CQ and insoluble particles respectively for a range of monomers.

Monomer mixtures were weighed using an electronic four-figure analytical balance, and stored in brown glass bottles to prevent premature light activation. A magnetic stirrer bar was used to aid mixing. Monomers were left to mix at room temperature until no further change could be detected. These were then kept in the fridge for at least 24 hours before they were assessed or used.

The levels of components for 4 groups of monomers A-D is provided in Table 2-3. The first aim was to determine whether using an accelerator such as DMAEMA could affect solubility of the solid monomer 4META (a monomer that is used to improve adhesion) in liquids containing UDMA and either PPGDMA (group A) or TEGDMA (group B). The ratio of the bulk monomer UDMA to the diluent monomers PPGDMA or TEGDMA was 3:1. In monomer groups A and B the concentrations of the 4 META and CQ were fixed at 5 wt% and 0.75 wt% respectively. The DMAEMA was used at three different concentrations (0, 1 and 1.5 wt%). In the third group of monomers (group C) UDMA and TEGDMA or PPGDMA were again used at 3:1. 4META and DMAEMA was fixed at 5 wt% and 1 wt% whilst CQ was 0.5 or 1 wt%. In group D, liquid phases were prepared with UDMA: PPGDMA at 3:1 or 0, 4META was reduced to 3 wt%, DMAEMA removed and CQ used at 0.5 or 1 wt%.

	UDMA Base monomer	**PPGDMA Diluent monomer**	**TEGDMA Diluent monomer**	**4META Adhesive monomer**	**DMAEMA Activating monomer**	**CQ Initiator**	**Total**
Monomer	(wt%)						
A1	71	23.25	0	5	0	0.75	100
A2	70	23.25	0	5	1	0.75	100
A3	70	22.75	0	5	1.5	0.75	100
B1	71	0	23.25	5	0	0.75	100
B2	70	0	23.25	5	1	0.75	100
B3	70	0	22.75	5	1.5	0.75	100
C1	70	0	23	5	1	1	100
C2	70	0	23.5	5	1	0.5	100
C3	70	23	0	5	1	1	100
C4	70	23.5	0	5	1	0.5	100
D1	96	0	0	3	0	1	100
D2	96.25	0	0	3	0	0.75	100
D3	72	24	0	3	0	1	100
D4	72	24.25	0	3	0	0.75	100

Table 2-3 Percentages of bulk diluent and adhesion promoting monomers and wt% of activator and photoinitiator in monomer series A, B, C and D

2.11 Preparation of composite pastes and assessment of monomer conversion

Basic composite pastes (Table 3-4 and 3-5), with a powder to liquid weight ratio of 4:1 (wt/wt), were prepared from all monomer groups A-D using an automatic centrifugal mixer and airtight bottles (Speedmixer, Hauschild Engineering) at a speed of 3500 rpm for two minutes. Filler phase was fixed as 7 micron glass only for A-C series. In addition, series D formulations were prepared using 7 micron glass with and without 2% PLS added. The pastes were stored in dark containers at room temperature of 21-23 ^{o}C before assessment of monomer conversion and reaction rate as described in section 2.3.

Composite	Glass	PLS %	MCPM %	PLR	Test
		Wt%			
A1	7µm	0	0	4	FTIR
A2	7µm	0	0	4	FTIR
A3	7µm	0	0	4	FTIR
B1	7µm	0	0	4	FTIR
B2	7µm	0	0	4	FTIR
B3	7µm	0	0	4	FTIR
C1	7µm	0	0	4	FTIR
C2	7µm	0	0	4	FTIR
C3	7µm	0	0	4	FTIR
C4	7µm	0	0	4	FTIR

Table 2-4 Composite groups A, B, C and D with the type of glass used, PLS wt%, MCPM wt%, PLR and the type of experiment

Composite	Glass	PLS %	MCPM %	PLR	Test
D1 or D1'	7µm	0 or 2	0	4	FTIR
D2 or D2'	7µm	0 or 2	0	4	FTIR
D3 or D3'	7µm	0 or 2	0	4	FTIR
D4 or D4'	7µm	0 or 2	0	4	FTIR

Table 2-5 antibacterial containing composites

2.12 Preparation and characterization of composite discs with varying MCPM and PLS:

3.4.1 Preparation and composition of discs

Discs were prepared with filler compositions given in Table 2-6. To prepare the composite discs, monomer D3 was combined with a hybrid glass consisting of 7 micron, 0.7 micron and Nano silica particles in the weight ratio of 6:3:1. In F1 composites, MCPM and PLS were added to the filler phase at levels of 8 and 2 wt% respectively. In F2 composites MCPM and PLS were added at 4 and 5 wt% respectively. Both formulations were mixed with D3 monomer at powder to liquid ratios of 5, 4 and 3 using methods described above. Four control composites were prepared with hybrid glass and MCPM 0,4 or 8 wt% and PLS at 0, 2 and 5wt%. In addition the commercial material Z250 was examined. Its composition is given in Table 2-7.

Formula	Monomer	Glass	PLS %	MCPM %	PLR	Test
F1/3 F1/4 F1/5	D3	Hybrid	2	8	3 4 5	Mass & volume + spectroshade + HPLC
F2/3 F2/4 F2/5	D3	Hybrid	5	4	3 4 5	Mass & volume+ spectroshade + HPLC
F3/8 F3/4	D3	Hybrid	0	8 4	3	Mass & volume
F4/2 F4/5	D3	Hybrid	2 5	0	3	Mass & volume

Table 2-6 Formulations used in polylysine release, mass, volume and colour change studies

Name	Supplier	Batch No/code and date	Monomers	Powder
Filtek	3M ESPE, Deutschland	November/2016	Bis-GMA UDMA BIS-EMA	Zirconia/silica 0.01 – 3.5 µm

Table 2-7 Components of the commercial composite material (Z250)

Disc samples were prepared by placing the pastes in metal rings of 1 mm depth and 10 mm internal diameter. An acetate sheet was used to cover the pastes top and bottom and pressed to remove the air. Maximum polymerisation of the composite discs was obtained by curing both sides of the composite disc for 40 seconds at different points. Composite discs were polished using a silicon carbide (500) paper.

1.1.2 Mass and volume change in water, for composite materials containing different percentages of MCPM and PLS

Three samples were made of each of the six formulations. Mass and volume change in water were then assessed for eight weeks as discussed in section 2.6.

1.1.3 Water sorption and solubility

Following eight weeks of immersion in water samples of F1 and F2 (see table 3-6) were dried for two weeks and water sorption as well as particle solubility were calculated using the following equations:

$$\text{Water sorption } (\mu g/mm3) = \frac{M(wet) - M(dry)}{V\ (intial)} \quad \text{Equation 5}$$

Solubility was calculated using the equation below:

$$\text{Solubility } (\mu g/mm3) = \frac{M(initial) - M(dry)}{V\ (intial)} \quad \text{Equation 6}$$

2.12.1 Colour evaluation experiments

To assess colour stability in different solutions composite discs of formulations F1/3, F2/3, F1/4, F2/4, F1/5, and F2/5 were immersed in coke (The Coca-Cola Company, tea (Essential Waitrose Original Blend tea, round bags), and red wine (Il Venti, Vino Rosso, italian red wine). for 1 week. Samples were then washed and dried with paper towel. Samples were photographed under daylight against the vita shade guide pre and post brushing using an electronic brush (see figure 3.5) Aquafresh® toothpaste was used with the electronic tooth brush for 30 seconds. An electronic balance was used to insure that equal brushing forces are applied throughout. To stabilize the composite disc during brushing, a putty impression material (Vinyle Polysiloxane, 3M Express™ STD) was used, covered with a plastic as shown below.
This evaluation is further to be quantified using Spectroshade™.

The photographs were taken using (a Canon G16 camera).

Figure 2.11 Composite discs brushing to remove superficial debris, on the right, a composite disc soaked in coke before brushing.

Immersion solutions

The first solution used was Coke (The Coca-Cola Company) at room temperature. The second solution was tea, prepared by immersion two tea bags (Essential Waitrose Original Blend tea, round bags), into 250 ml of boiled water, then left to cool at room temperature for 20 minutes, and the third solution was red wine (Il Venti, Vino Rosso, italian red wine). Composite discs were immersed in 1mm of the solutions and were stored in an incubator at 37˚ C for a week. The same experiment was repeated for the commercial dental composite.

The commercial composite discs were also immersed in in the different solution to solutions for a week to compare the effect with the experimented composite discs. Since it was noticed that some immersed composites reacted with coke; the other control composite discs were immersed in coke to check if the resulted effect seen in figure 4.21.

Visual Shade evaluation

Four dentists were asked to give a shade to the composite discs before and after immersion using the vita classical shade guide (VITA, H. Rauuter GmbH and co. KG, Germany) see figure 10 below, at 3 different times. This experiment was to check if there is an agreement between the most common method of shade measuring(direct vision) and the spectroshade which is regarded as a relaibale and objective method. Then using the vita classical shade guide, the change in colour was evaluated to see if

colour change could be visually noticed, before using the spectroshade. The four dentists from the paediatric department were evaluated the composite discs at normal clinical light.

To check reproducibility, dentists were asked to select a shade at three different occasions, the shade selected most was regarded as the shade of the disc.

The most frequently selected shade was taken to be the shade of the composite disc.

Figure 2.12 Vita classical shade guide commonly used by clinicians.

Later on the shade tabs on the Vita shade guide were arranged from lightest to darkest according to value. This is an arrangement recommended by the vita classical website and is described as a commonly used method for comparing colour, as below.

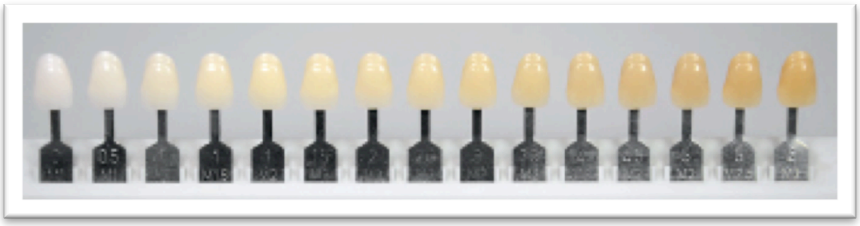


Figure 2.13 vita classical tabs arranged in terms of value (brightest to darkest, B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4).

A key was developed to represent the shades for easier representation of results as below the larger the number the darker the shade.

B1	1
A1	2
B2	3
D2	4
A2	5
C1	6
C2	7
D4	8
A3	9
D3	10
B3	11
A3.5	12
B4	13
C3	14
A4	15
C4	16

Table 2-8 Key for using the vita shade arranged in values.

Chapter Three

Results

3 Results

3.1 Visual assessment of monomer clarity

UDMA monomers containing PPGDMA (A1) or TEGDMA (B1) and 5 wt% 4META but no DMAEMA were initially cloudy (Fig 3.1a and Table 3-1). B1 became clear after 24 hours due to sedimentation of the precipitate. Additions of either 1 wt% (A2 and B2) or 1.5 wt% (A3 and B3) of DMAEMA to either of these monomers lead to 4META dissolution and solutions becoming clear (Figure 3.1b). All monomer liquids of group C (containing 1 wt% DMAEMA) were clear. Monomer solutions of group D were also clear, despite containing 0 wt% DMAEMA, however, the amount of 4META was limited to 3 wt%. For all the liquids the amount of CQ was variable. Differences in colour were not detectable by eye. All solutions appeared yellow.

a **b**

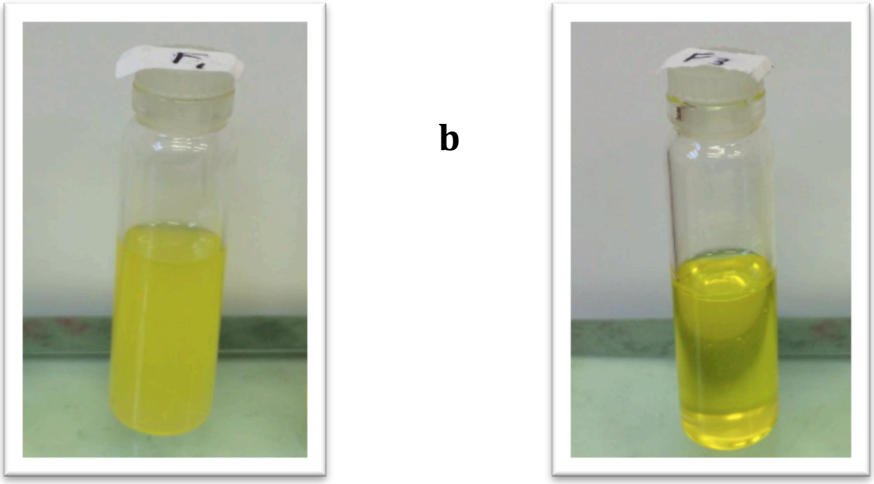


Figure 3.1 a and b, UDMA/PPGDMA monomers with 0.75 wt% CQ, 5 wt%4META and a) 0 wt% DMAEMA (A1) or b) 1 WT% DMAEMA

Monomer liquids	Diluent monomer	4META Adhesive monomer	DMAEMA Activating monomer	CQ Initiator	Clarity
	(wt %)				
A1	PPGDMA	5	0	0.75	Cloudy
A2	PPGDMA	5	1	0.75	Clear
A3	PPGDMA	5	1.5	0.75	Clear
B1	TEGDMA	5	0	0.75	Cloudy
B2	TEGDMA	5	1	0.75	Clear
B3	TEGDMA	5	1.5	0.75	Clear
C1	TEGDMA	5	1	1	Clear
C2	TEGDMA	5	1	0.5	Clear
C3	PPGDMA	5	1	1	Clear
C4	PPGDMA	5	1	0.5	Clear
D1	None	3	0	1	Clear
D2	None	3	0	0.75	Clear
D3	PPGDMA	3	0	1	Clear
D4	PPGDMA	3	0	0.75	Clear

Table 3-1 Monomer compositions in formulations A, B, C and D and solution clarity

3.2 UV spectra of monomers

The UV spectra of monomers or supernatant above sediment gave peak absorbance at 470 nm (Figure 3.2, 3.3 and 3.4). The UV spectra of cloudy suspensions, however, (e.g. A1 in Figure 3.3) had an apparent high background absorbance due to light scattering in addition to the CQ absorbance peak. Absorbance of monomer solutions of A2, A3, B1 (supernatant), B2 and B3 all superimposed as they contained the same amounts of CQ and were clear (see figure 4.3).

Group C liquids contained 1 wt% DMAEMA and were all clear (Table 3-1 and Figure 3.4) and so gave no background scattering in the UV spectra UV spectra of group D are shown in figure 4.5. In this group a reduced amount of 4META was used (3 wt%). As can be seen from Figure 3.5 and table 3-1 all monomer liquids were clear with 3% 4META even without activator addition.

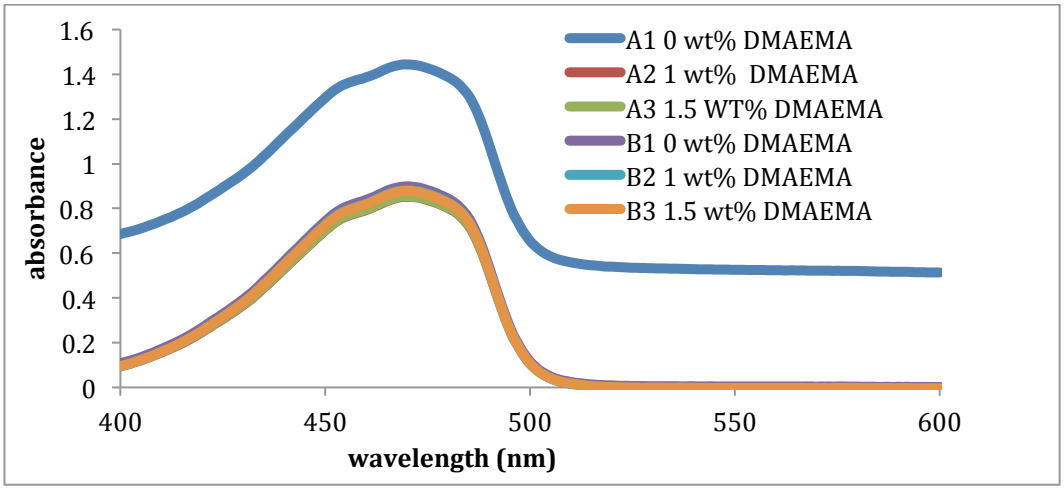


Figure 3.2 UV-visible spectra of group A and B monomers containing 23 wt% PPGDMA versus TEGDMA respectively. 4META and CQ are fixed at 5 and 0.75 wt% whilst DMAEMA is 0, 1 or 1.5 wt%.

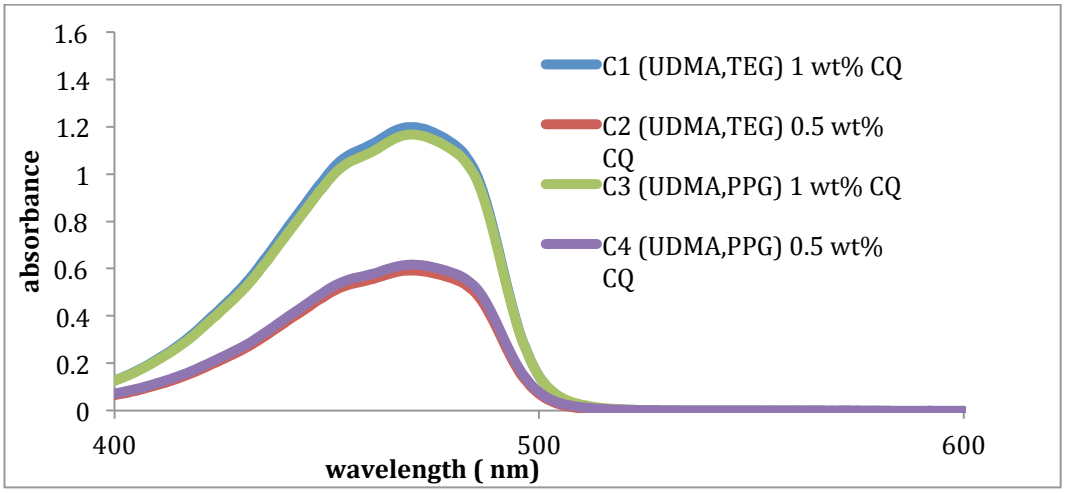


Figure 3.3 UV visible light spectra of group C monomers containing 23 wt% PPGDMA versus TEGDMA respectively. 4META and DMAEMA are fixed at 5 and 1 wt% whilst CQ is 1 or .5 wt%.

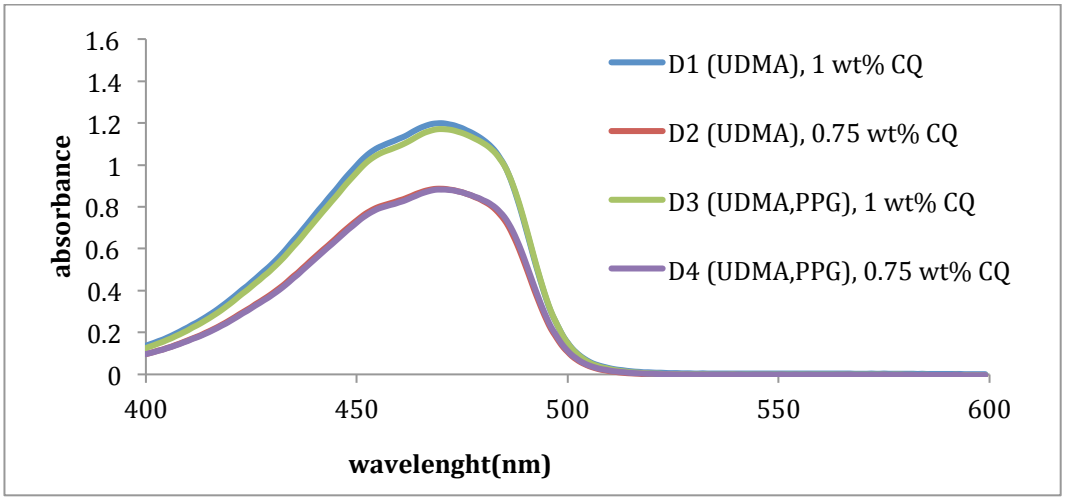


Figure 3.4 UV visible light spectra of group D monomers containing either UDMA or UDMA with PPGDMA at 24 wt%. 4META is fixed at 3 wt% whilst CQ is 1 or .75 wt%.

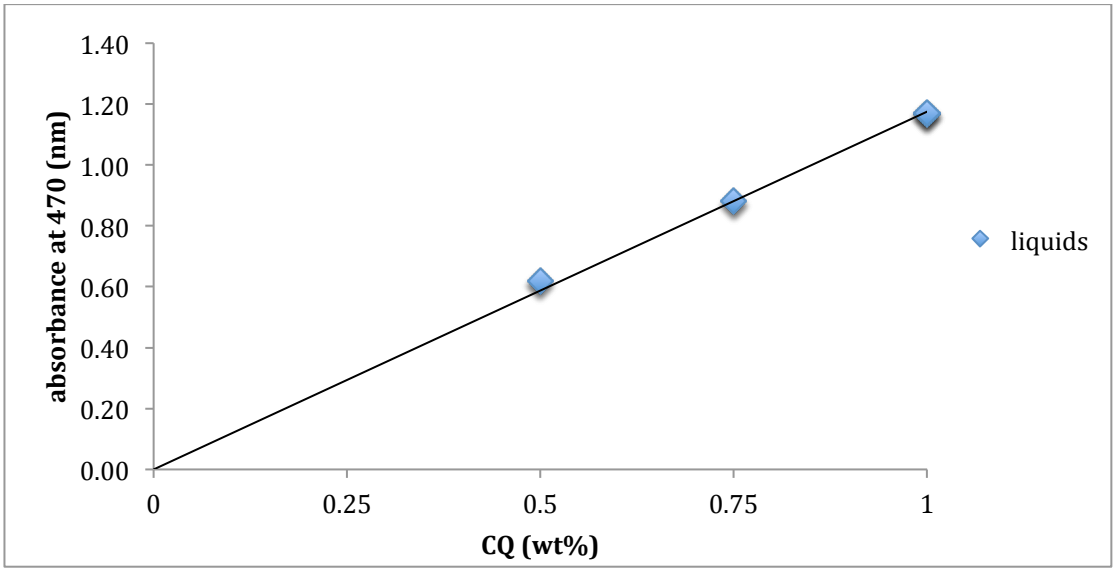


Figure 3.5 the graph represents the absorbance at 470 (nm) of monomers A, B, C and D at different wt% of CQ (05, 0.75 and 1), formulations A and B all contained 0.75 wt%, C1 and C3 contained 1% CQ and either TEGDMA or PPGDMA respectively. C2 and C4 contained 0.5 wt% CQ and either TEGDMA or PPGDMA respectively. The cloudy solution A1, did not fit the trendline and therefore not added.

3.3 FTIR analysis of basic composites

3.3.1 Basic composite polymerisation rates

Figure 3.6 shows polymerisation rates calculated from FTIR results. Conversion rates decrease as DMAEMA is increased in composites of group A. For composites containing PPGDMA in the liquid phase, maximum conversion rate of 5.17 s was achieved when DMAEMA was 0 wt%. Upon increasing the concentration of DMAEMA to 1 and then 1.5 wt% this declined to 4.7 and then 4.0 %/s. Conversely, with TEGDMA, maximum reaction rate increased with increasing DMAEMA (from 2.99 to 5.07 %/s).

Figures 4.6 and 4.7 show polymerisation reaction rates of composite series C and D. the effect of varying the amount of CQ and PLS on reaction rates is minimum with all results ranging from 3.4 to 4.3.

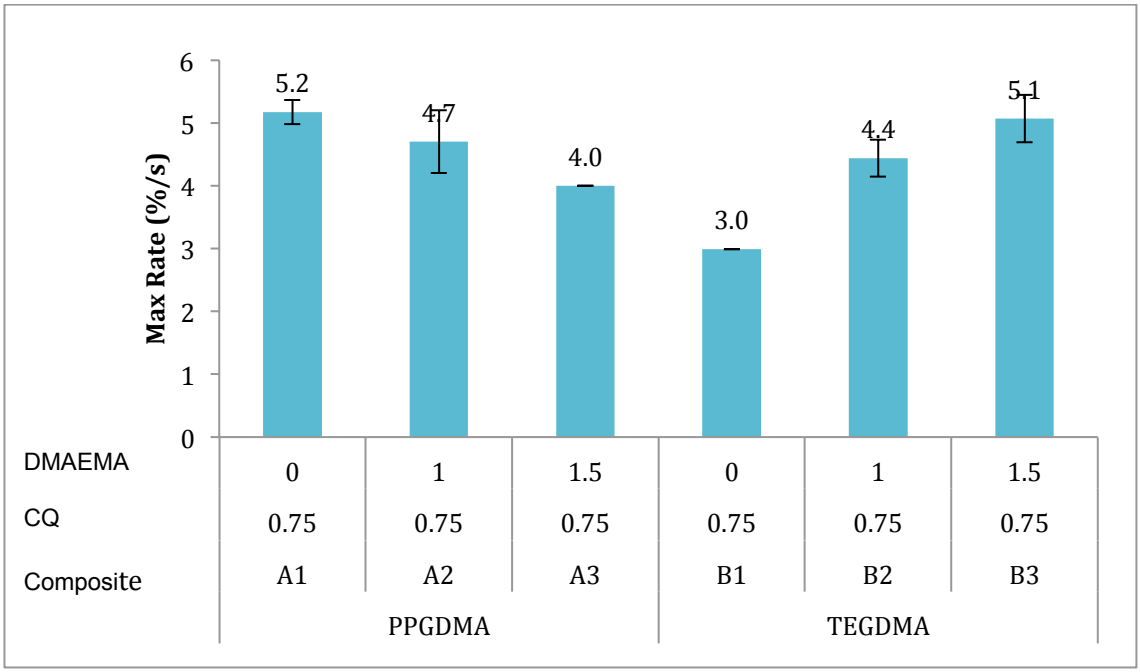


Figure 3.6 Maximum rate (%/s) of composite groups A and B monomers containing 23 wt% PPGDMA versus TEGDMA respectively. 4META and CQ are fixed at 5 and 0.75 wt% whilst DMAEMA is 0, 1 or 1.5 wt%, error bars= SD, n=3.

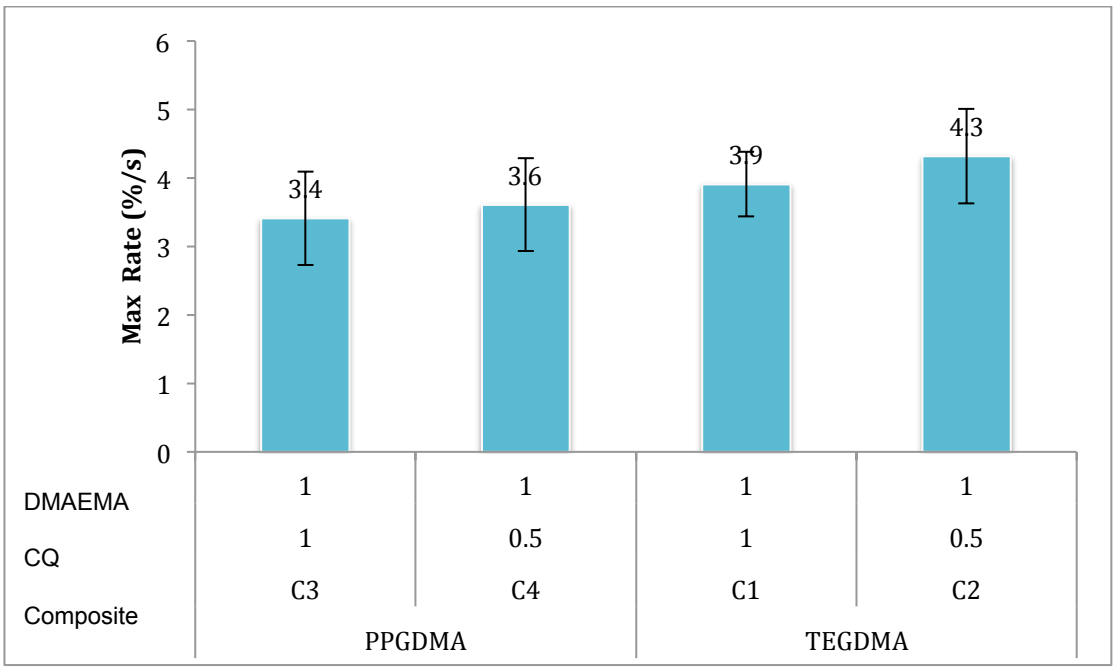


Figure 3.7 Maximum rate (%/s) of group C monomers containing all UDMA and 23 wt% PPGDMA (C3 and C4) versus TEGDMA (C1 and C2) respectively. 4META and DMAEMA are fixed at 5 and 1 wt% whilst CQ is 1 or 0.5 wt%, error bars= SD, n=3.

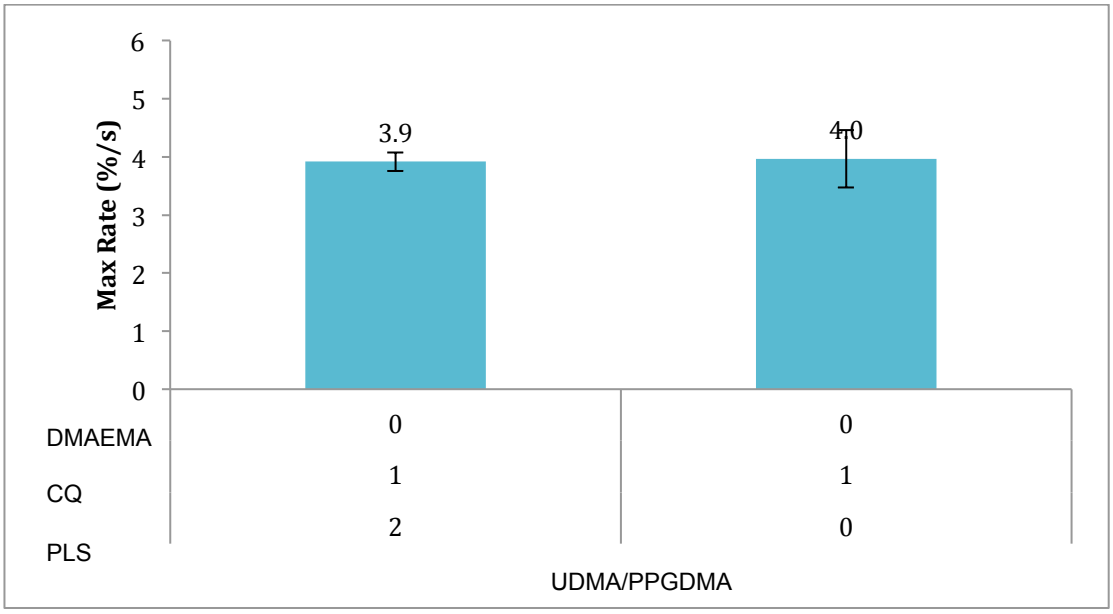


Figure 3.8 Maximum rate (%/s) of D3 containing UDMA with PPGDMA with and without 2 wt% PLS, both containing 3 wt% 4META and 0 wt% DMAEMA, error bars= SD, n=3.

3.3.2 Final monomer conversions

Series A and B basic composites

Figure 3.9 gives the monomer conversions for A and B series composites produced using 7 micron glass as the filler phase, PPGDMA versus TEGDMA diluent monomer and varying DMAEMA.

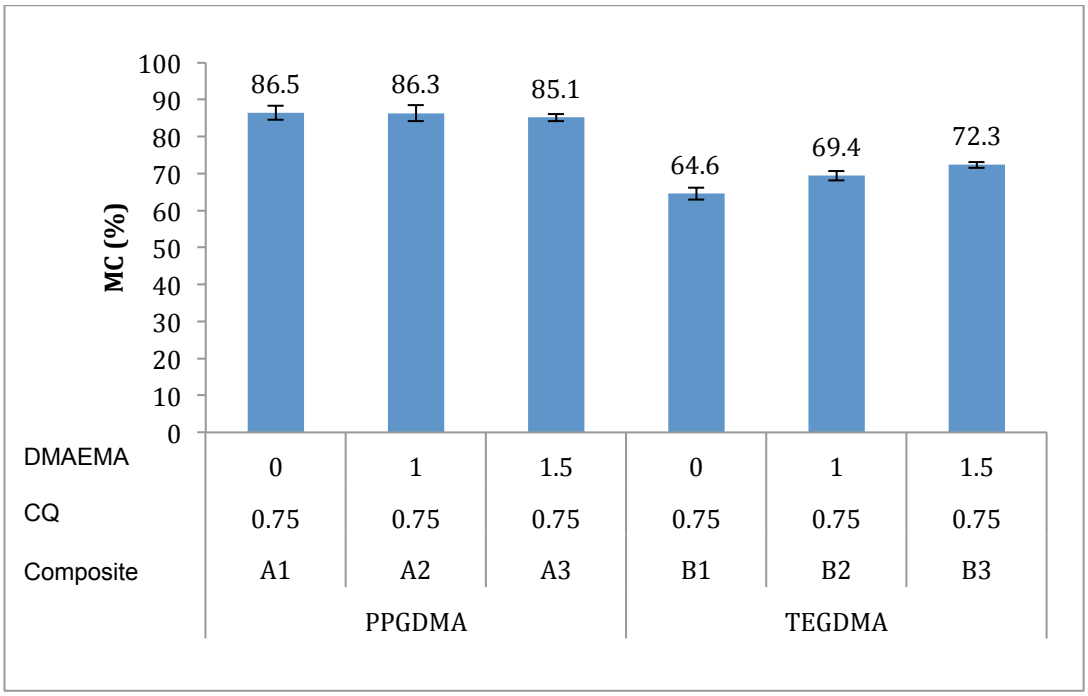


Figure 3.9 MC % of group A and B monomers containing UDMA and 23 wt% PPGDMA versus TEGDMA respectively. 4META and CQ are fixed at 5 and 0.75 wt% whilst DMAEMA is 0, 1 or 1.5 wt%, error bars= SD, n=3.

Composites A1 to A3 containing PPGDMA have monomer conversion ranging from 82 to 84 %. Composite A1 had a high monomer conversion although the monomer liquid was not clear due to undissolved 4META. Composites A2 and A3 contained 1 and 1.5 wt% of DMAEMA and were both clear (see table 4-1). The added DMAEMA, however, gave no significant increase in degree of monomer conversion.

Composites B1-B3 with TEGDMA as a diluent monomer had significantly lower monomer conversion compared to the ones containing PPGDMA. With these composites the average degree of monomer conversion ranged from 69.5% to 62.5%. As with composites of group A; the addition of DMAEMA helped in clarifying the monomers. It also, however, slightly increased monomer conversion of the B series composites.

Series C basic composites

For the C series composites, CQ of 0.5% resulted in higher monomer conversion than 1% irrespective of whether the diluent was PPGDMA or TEGDMA (Figure 3.10). Additionally, the MC% of C series composites containing PPGDMA was considerably higher than with those containing TEGDMA. Comparing with the A and B series composites, C composites with TEGDMA and 1 wt% DMAEMA had reduced monomer conversion when CQ was decreased from 0.75 (in B2) to 0.5 wt% (C2) or increased to 1 wt% (C1). The decline was more significant with the higher CQ level. A similar trend was obtained upon comparing composites A2 with C4 and C3. All contain PPGDMA, 1 wt% DMAEMA but CQ of 0.75, 0.5 and 1 wt% respectively.

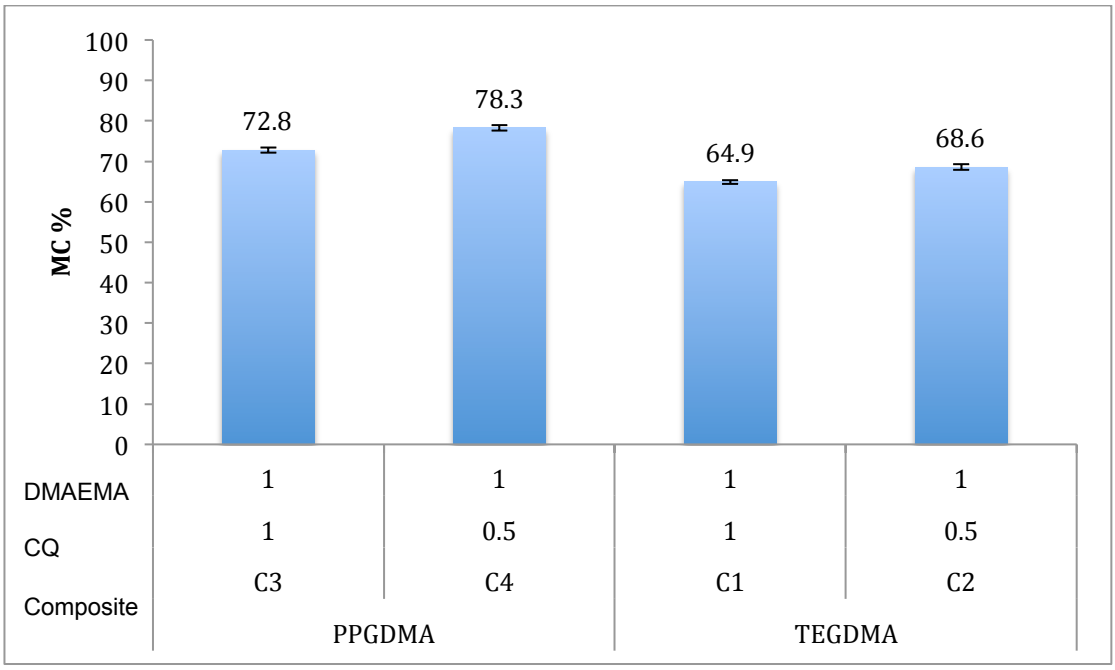


Figure 3.10 MC % of C1 to C4 (n = 3), monomers containing 23 wt% PPGDMA versus TEGDMA respectively. 4META and DMAEMA are fixed at 5 and 1 wt% whilst CQ is 1 or .5 wt%, error bars= SD, n=3.

Composites D1-D4 (The effect of adding PLS on monomer conversion)

Eight composites were prepared using monomer liquids of group D with either glass (7 µm) alone or glass with 2% PLS. Figure 3.11 provides a comparison of monomer conversions; refer to table 3-1 for monomer formulations.

From Figure 3.11, composites with no diluent monomer or DMAEMA have a lower MC% compared with formulae with 25% PPGDMA as a diluent monomer, irrespective of CQ. The average MC% obtained for formula D1 (containing 1 wt% CQ, 0 wt% PLS and 3 wt% 4META) and no diluent was 56.3%. This increased slightly with the addition of PLS to reach 60%. Composites D2 (0% PPGDMA, 3 wt% 4META, 0 wt% DMAEMA) the MC% were 56.4 and 57.7%, with and without PLS

Composites containing D3 and D4 with added PPGDMA monomer conversions were much higher compared with formulations D1 and D2. There was no significant difference in MC% between D3 and D4 although D4 contained a reduced amount of CQ (0.75 wt%).

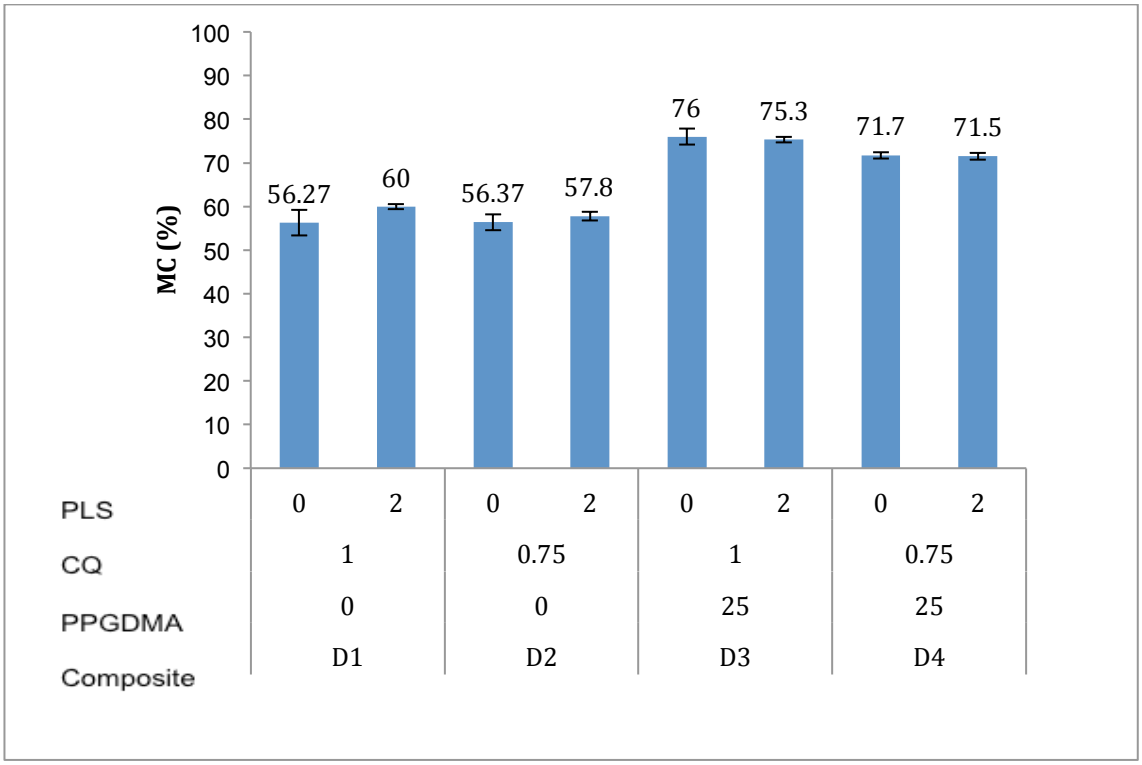


Figure 3.11 MC % of group D, monomers containing either UDMA or UDMA with PPGDMA at 24 wt%. 4META is fixed at 3 wt%, DMAEMA has been removed whilst CQ is 1 or .75 wt%, error bars= SD, n=3.

Univariate analysis of variance on SPSS (SPSS statistics version 24, IBM, USA) was used to analyse the effect of variables individually. Three samples were used in each experiment. Results may be improved by using a large sample size.

To investigate for significance of the results obtained variables were set as Table 3-2 below, and the effect of each was tested against the MC% results.

Variable 1	PPGDMA or TEGDMA
Variable 2	CQ wt% (0.5, .75 or 1 wt%)
Variable 3	DMAEMA wt% (0, 1 or 1.5%)

Table 3-2 variables tested on SPSS against MC%

One way analysis of variance (ANOVA) was used to check the effect of each variable on monomer conversion (MC %). On normal distributed data, there was a statistically significant result for the effect of using monomers with either PPGDMA or TEGDMA on MC%, with *p*-value of .000. There was also statistically significant results obtained for the effect of CQ wt% on MC%, *p*-value less than .05. According to the statistical analysis (see appendix 1) 94% of the results and changes in MC% can be attributed to the use of PPGDMA or TEGDMA and the concentration of CQ.

3.4 Properties of active composite discs

3.4.1 Appearance of composite discs

Composite discs containing CQ wt% appeared yellow before curing. Photobleaching, however, following 40 seconds of blue light curing resulted in a visible improvement, as noted in Figures 3.12 a) and b).

a

b

Figure 3.12 Example composite discs a) before and b) after curing

3.4.2 Mass and volume change of composite discs in water

Mass change in water of formulations F1 and F2

In Figure 3.13 below, the formulations are coded F1 or F2 according to the percentage of MCPM and polylysine followed by a number that indicates the PLR. As can be seen from the graph, in general mass change is proportional to the square root of time

initially but then levels off after about 1 to 2 weeks. Formulations F1, with a higher amount of polylysine and less MCPM have a higher mass change percentage than those of F2 irrespective of PLR. On raising PLR, mass change first decreased and then increased having a minimum value with PLR 4 for both F1 and F2 formulations. The highest mass change % was obtained from formulation F1/3, with 5 wt% polylysine and a PLR of 3. Lowest mass change was with composite F2/4.

F1/3, containing 4 wt% MCPM and 5 wt% polylysine, had a mass change of 4.08 % at the fourth week. F2/3 with 8 wt% MCPM and only 2wt% polylysine, had a maximum mass change at week four of 1.8%. With a PLR of 4, F1/4 had a maximum mass change of 2.7% whilst F2/4 final mass change was 1.27%. Mass change for F1/5 was 3.1%, while it was only 1.9% for F2/5.

Volume change in water of formulations F1 and F2

Volume change against square root of time of F1 and F2 with PLR (3,4 and 5) are presented in figure 4.14. Generally, there was a gradual but slow increase in volume over time; F1/4 composites have the highest volume change of 2.37% while F2/5 have the lowest about 1.4%.

Mass change in water

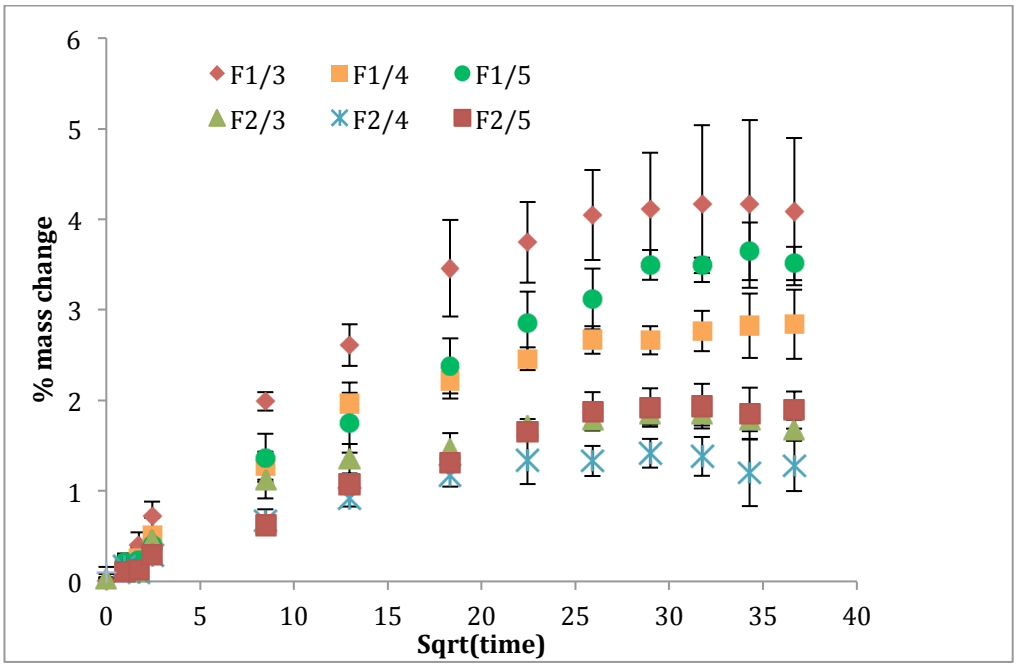


Figure 3.13 % mass change in water / square root of time of formulations F1 and F2 o with different powder liquid ratios for eight weeks (n = 3), F1 and F2 containing UDMA, PPGDMA. F1 contains MCPM and PLS at 4 and 5 wt% and F2 contains 8 and 2 wt%, the number 3,4 and 5 represent the powder liquid ratio. Error bars= SD, n=3.

Volume change in water

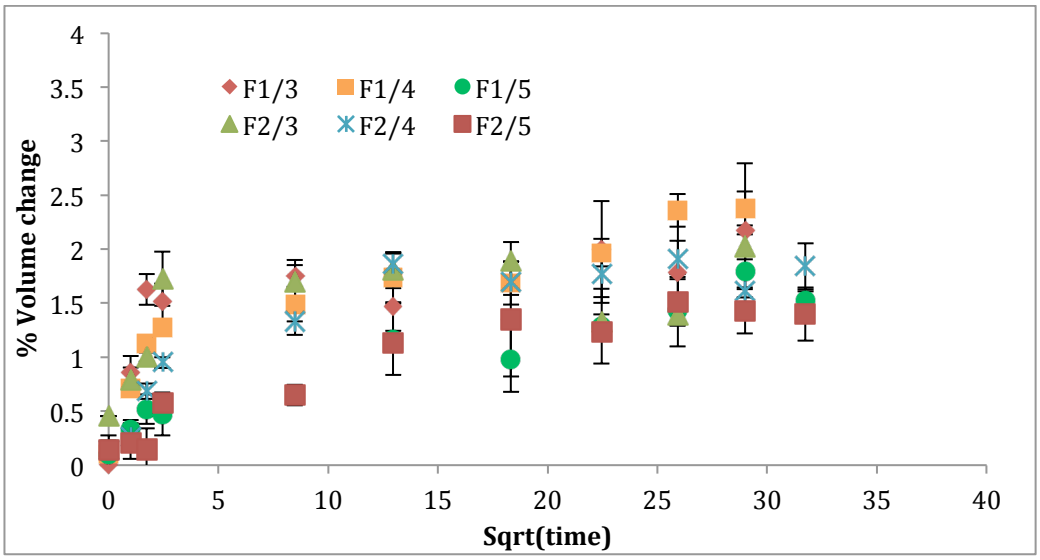


Figure 3.14 % volume change in Water versus Square root of time of formulations F1 and F2 of different powder liquid ratios for eight weeks (n = 3), F1 and F2 contain UDMA and PPGDMA. F1 contains 4 and 5 wt% and F2 contains 8 and 2 wt% MCPM and PLS respectively. The number 3,4 and 5 indicates the powder liquid ratio. Error bars= SD, n=3.

3.5 Mass and volume changes of controls in water

Figures 3.15 and 3.16 provides results of mass and volume changes of a commercial composite and formulations with different amounts of PLS and MCPM against the square root of time. Z250 (commercial composite) have the lowest change in both mass and volume compared to the other controls. The highest mass change was with 5 wt% PLS (5 and 2 wt%) which was 4.56 % and 2.5% respectively. MCPM 8 and 4 wt% resulted in a minimum increase in mass.

Volume changes were lower for commercial composite but higher with composites containing 5% PLS.

Mass change in water

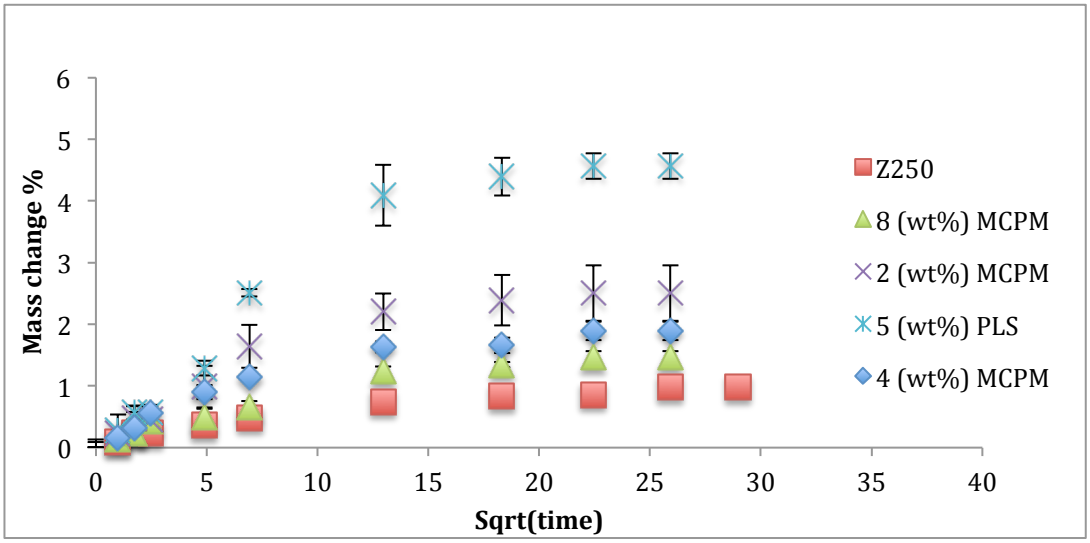


Figure 3.15 Mass change in water of controls, Z250 (commercial composite), two control composites with MCPM 8 and 4 (wt%) without PLS and two control composites with PLS 5 and 2 (wt%) without MCPM. Error bars= SD, n=3.

Volume change in water

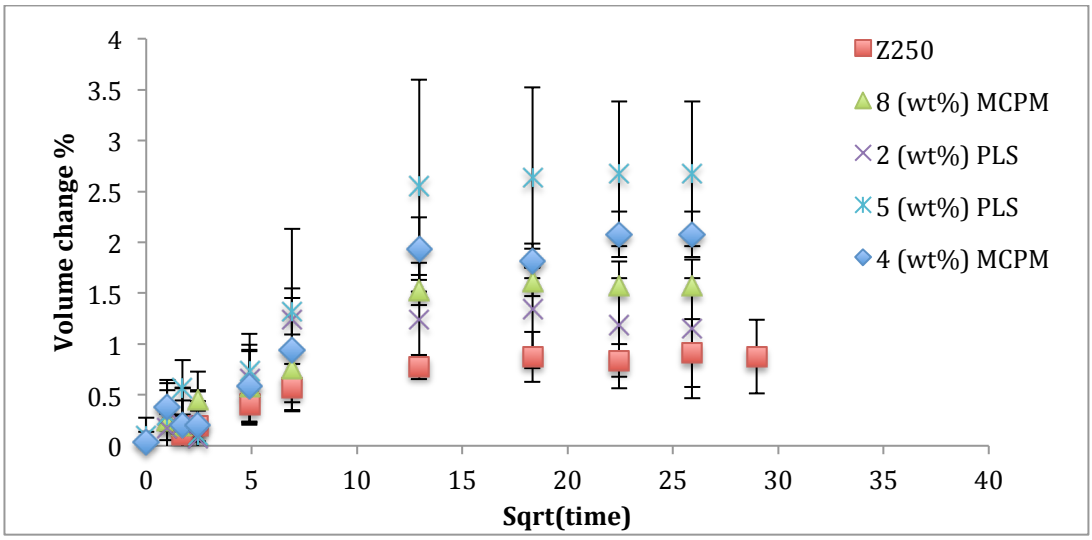


Figure 3.16 Volume change in water of controls, Z250 (commercial composite), two control compo sites with MCPM 8 and 4 (wt%) without PLS and two control composites with PLS 5 and 2 (wt%) without MCPM. Error bars= SD, n=3.

Distribution of results was normal (see appendix 1) for average mass% values; the effect of formulation (F1 and F2, from table 3-6) was tested against the results obtained for mass and volume percentage changes as well as the effect of varying the ratio or PLR using (ANOVA). Statistical significant results were obtained for all the variables with *p*-value below .000.

With regard to volume% change, the results were also of normal distribution (appendix 1). Analysis of the results using (ANOVA) suggested significant results in terms of PLR with *p*-value below 0.05. There was no statistically significant result in relation to the formulations i.e. (F1 AND F2 see table 3-6), bigger sample size would probably be more reliable. Table 4-3 shows the variables for statistics,

Variable 1	F1 or F2
Variable 2	PLR (3,4 or 5)

Table 3-3 The table showing variables tested for mass and volume change using SPSS .

3.6 Water sorption and solubility

Water sorption and solubility of F1 and F2 series with PLR of 3,4 and 5 are shown in Figures 3.17 and 3.18 respectively. On average water sorption of F1 formulations with higher PLS and lower MCPM is greater than that for F2 composites. Solubility,

however, is lower with higher PLR. Maximum water sorption was achieved with formulation F1/4 with a value of 112.1 µg/mm3.

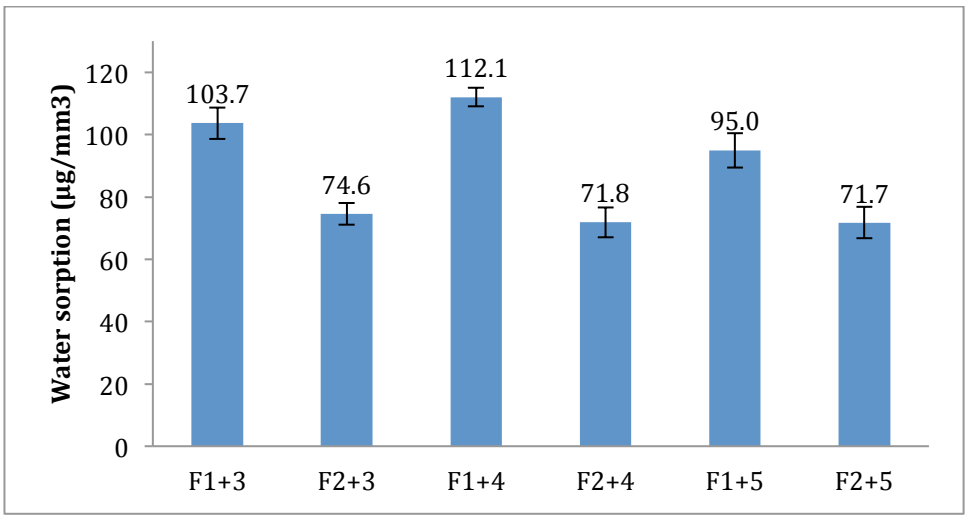


Figure 3.17 water sorption of formulations F1 and F2 after eight weeks of immersion in water. Both F1 and F2 contain UDMA and PPGDMA, F1 contains 2 wt% of PLS and 8 wt% of MCPM, while F2 contains 5 wt% PLS and 4 wt% MCPM, the numbers 3,4 and 5 indicate the PLR

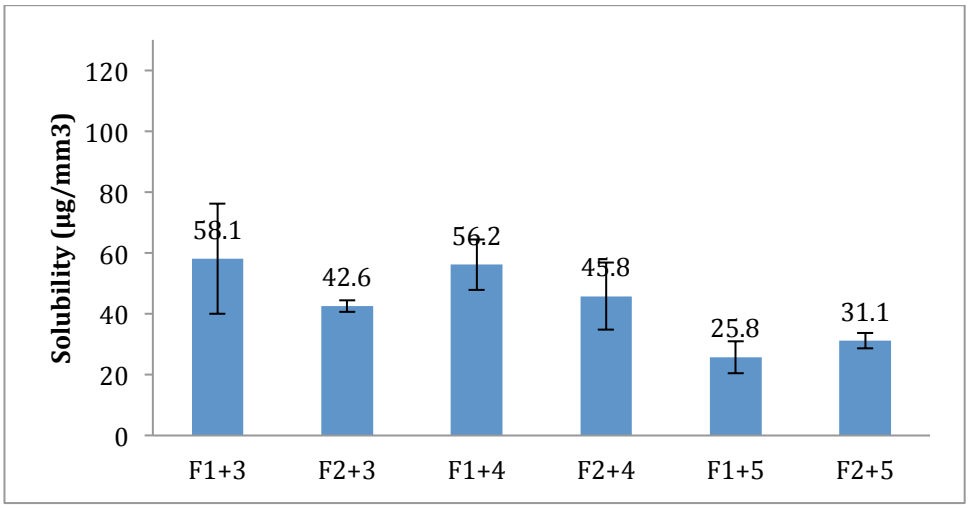


Figure 3.18 Solubility of particles of formulations F1 and F2 after eight weeks of immersion in water. Both F1 and F2 contain UDMA and PPGDMA, F1 contains 2 wt% of PLS and 8 wt% of MCPM, while F2 contains 5 wt% PLS and 4 wt% MCPM, the numbers 3,4 and 5 indicate the PLR.

3.7 Cumulative polylysine (PLS) release (8 weeks in water)

Figure 3.17 below represents accumulative PLS release (%) at two months for formulations F1 and F2, PLR 5 and 3 with discs stored in distilled water. As can be seen, in general formulations with PLS 5 wt% have higher PLS release. F1/5 had the highest cumulative release (16.7%) whilst F2 had the lowest release of 10.4 wt% for both PLR 5 and 3. F1/3 had an intermediate release of 13.7%.

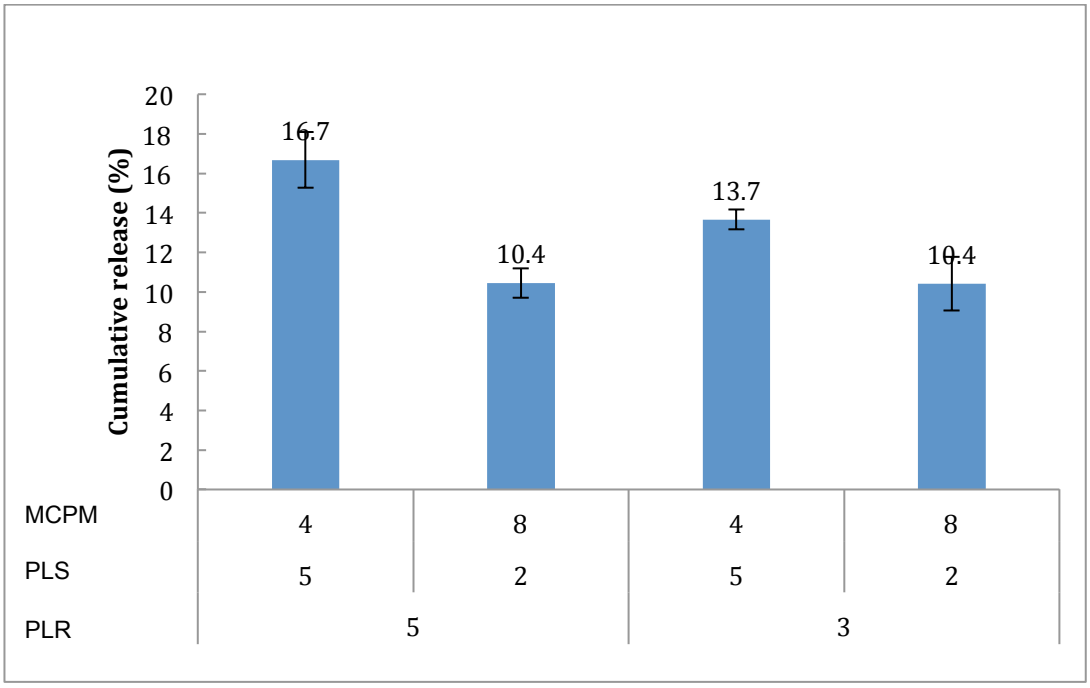


Figure 3.19 Cumulative polylysine (PLS) release for formulations F1/F2 PLR 5 and 3 for a period of two months after immersion in water, both F1 and F2 contain UDMA/PPGDMA and 3 wt% 4META (reproduced from Nabih Al khouri's data), error bars= SD, n=3.

3.8 Colour evaluation results

Visual Shade evaluation

Photographs of the composite discs before and after brushing are given in Figures 3.20 and 3.21 respectively. Shade as recorded by four different dentists is provided in Tables 3-4 a) and 3-5. It was observed that, red wine resulted in the worst discolouration in all of the composites.

F1+3

F2+3

F1+4

F2+4

F1+5

F2+5

Figure 3.20 Composite discs (by column first not immersed, then immersed in, coke, tea, and red wine for one week after and before brushing

	Not immersed	Coke	Tea	Red wine
F1/3	B1 (1)	B1 (1)	C1 (6)	D2 (4)
F2/3	B1 (1)	B1 (1)	C1 (6)	D2 (4)
F1/4	B1 (1)	B1 (1)	A2 (5)	C3(14)
F2/4	B1 (1)	B2 (3)	C1 (6)	C3(14)
F1/5	B1 (1)	B2 (3)	C1 (6)	C3(14)
F2/5	B1(1)	B1 (1)	B2 (3)	D2(4)

B1	**1**
A1	2
B2	3
D2	4
A2	5
C1	6
C2	7
D4	8
A3	9
D3	10
B4	11
A3.5	12
B4	13
C3	14
A4	15
C4	16

Table 3-4 a)shades as recorded by the dentists and b)the key, the larger the number, the darker the shade.

From Tables 3-4 and 3-5 it can be clearly seen that, red wine resulted in the worst discolouration. Despite that, if a comparison between the worst discolouration and the shades on the vita classical, it can be seen that the discolouration is not severe apart from with F1/5. Using the key provided, a D2 shade corresponds to number 4, which is, regarded a low value. However, the scores given also reflect the limitation of using the visual shade guide in that, the worst stained discs were recorded as either C3 with a number 14 on the key, or D2 which is a number 4. A score of C1 which is 6 on the key, was the most selected for discs immersed in tea, although if table 4-2 is compared to the figure 4.23 it can be seen that there are changes in colour again reflecting the limitation of using the visual methods of shade selection.

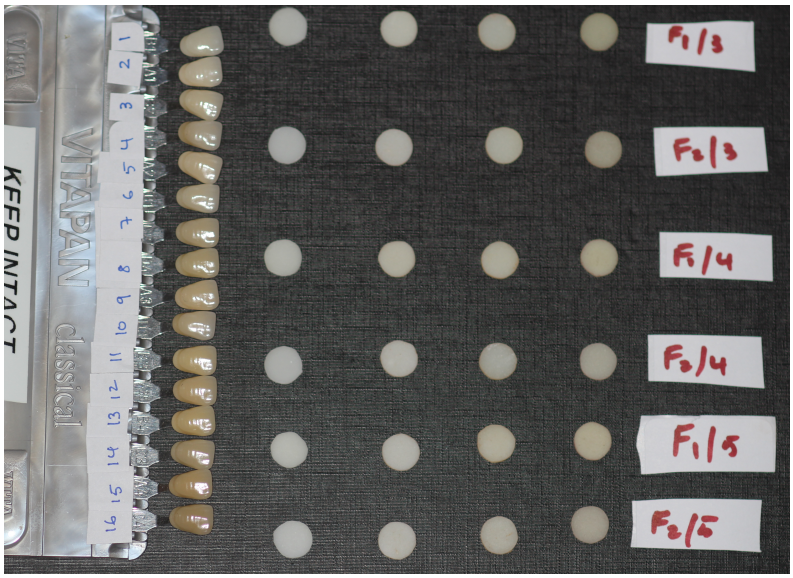


Figure 3.21 Composite discs after brushing (by column first not immersed, then immersed in, coke, tea, and red wine for one week) compared to vita classical shade guide with shades arranged in terms of value

	Not immersed	Coke	Tea	Red wine
F1/3	B1 (1)	B1 (1)	C1 (6)	D2 (4)
F2/3	B1 (1)	B1 (1)	B1 (1)	D2 (4)
F1/4	B1 (1)	A1 (2)	A1 (2)	D2 (4)
F2/4	B1 (1)	B1 (1)	C1 (6)	D2 (4)
F1/5	B1 (1)	B1 (1)	C1 (6)	C3(14)
F2/5	B1 (1)	B1 (1)	B2 (3)	D2 (4)

Table 3-5 Shades as selected by dentists, after brushing (refer to the key Table 3-4 b).

3.9 Spectroshade results

Using the non-immersed disc as a reference and the option compare from the software, shades were compared in terms of CIE Lch. A reference point was selected for average values from the non-immersed disc and colour change was calculated in relation to the reference point values of the the non immersed disc.

Three points (middle, up and down) were selected and compared to the reference point and an average ΔE was calculated, see Appendix 2. Figure 3.22 represents the total results.

Values of L did not greatly deviate from the reference value (see appendix 2) and differences were higher when c (chroma) and h (hue) were evaluated. This indicates that the degree of brightness was similar with regard to the composite discs while the concentration and the tone where different. As demonstrated below both formulae F1/5 and F2/5 with a PLR of 5 were stained most. The most solution that resulted in the highest ΔE value is red wine then tea then coke. The values of ΔE can be calculated using the software from the spectroshade by calculating the differences of Lch values or by using the equation below. Composites discs immersed in coke are less affected in all the formulations. Formulations with PLR 5 were discoloured most, however there was no great difference in terms of PLR and the results were not statistically significant, *p*-value > 0.00 see appendix section 8.4.

$$\Delta E = ((\Delta L)^2 + (\Delta c)^2 + (\Delta h)^2))^{1/2}$$ **Equation 7** (Schneider et al,. 2009).

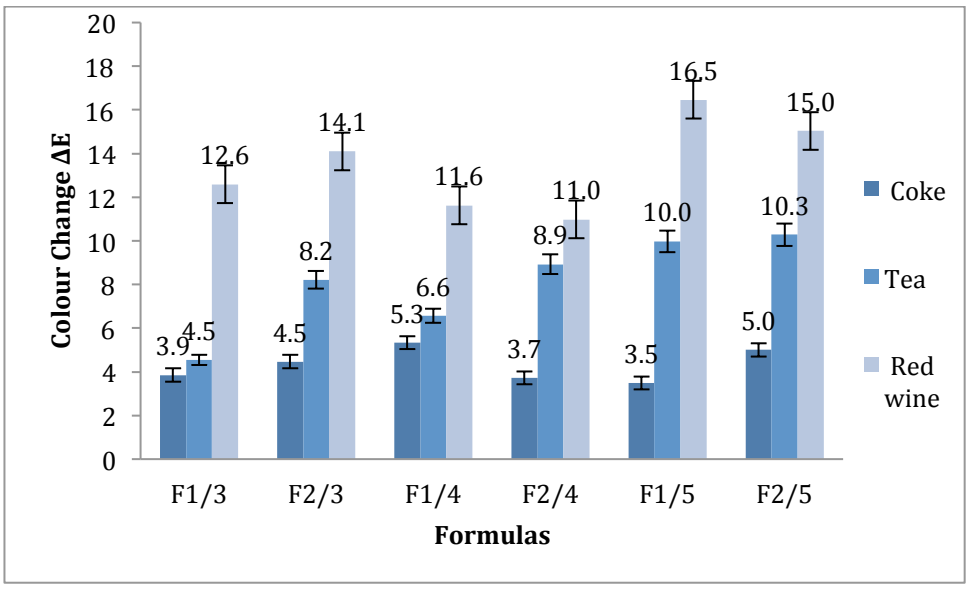


Figure 3.22 Average ΔE values obtained for discs immersed in different solutions compared to a non immersed disc of the same formulation (n = 3). F1 and F2 after one week of immersion in water. Both F1 and F2 contain UDMA and PPGDMA, F1 contains 2 wt% of PLS and 8 wt% of MCPM, while F2 contains 5 wt% PLS and 4 wt% MCPM, the numbers 3,4 and 5 indicate the PLR, error bars= SD,n=3

3.10 Commercial composite spectroshade results

Spectroshade was used to check the colour after brushing, figure 4.22 shows the results and the values of ΔE.

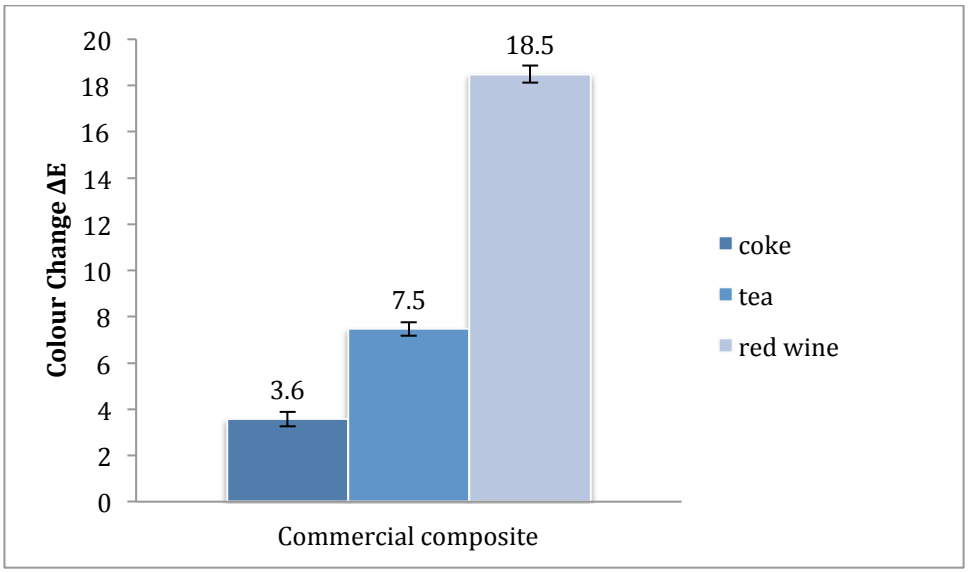


Figure 3.23 Average ΔE values obtained for discs of commercial material immersed in different solutions (n = 3) compared to a non immersed disc of z250 shade B1

It was observed that all composite discs immersed in coke resulted in a reaction, and on testing discs that contained eaither polylysine only or MCPM, it appeared that the reaction is a result of polylysine reacting with coke Figure 3.24. The relevance of this reaction is merely probably indicating the release of polylysine.

Figure 3.24 A composite disc containing 5 wt% PLS, 0 wt% MCPM, the effect of polylysine reaction with coke was observed, this was not observed when non-polylysine containing discs.

Chapter Four

Discussion

4 Discussion

Introduction

Composite filling materials are composed of two phases, the powder phase and the liquid or monomer phase. It is important that monomers are clear and homogeneous before composite materials are produced; therefore this study focused initially on the monomer content and by varying the wt% of the different monomers a clear ideal monomer matrix was produced. This is because monomers that are not polymerised may leach out of the restoration and result in cytotoxicity of the dentinal and pulpal cells (Schroeder and Vallo, 2007).

Different percentages of the activator DMAEMA were used to examine its effect on the clarity of monomers containing solid monomers such as 4META. It is common that photo-initiators such as CQ are combined with an amine in light cured materials such as composites (Musanje et al., 2009, Schneider et al., 2008). This is known as the binary system (Moon and Shin, 2012). CQ used at optimised levels affects the process of polymerisation positively (Yoshida and Greener, 1993). However, its bright yellow colour is a major drawback. There are many CQ concentrations that can be used in combination with an activator in order to improve polymerisation as demonstrated in the Schneider and Vallo study (Schroeder and Vallo, 2007).

In the initial phase of the research, clear monomers were mixed with one type of glass only, which is 7 μm to prepare the composite discs for checking the MC% using FTIR at a constant PLR of 4. The effect of varying DMAEMA and CQ wt% was also checked. Clarity of the monomers was assessed using UV spectrometry. In the later phase of the research DMEMA was eliminated from the monomer phase, this meant, however, that the amount of the solid monomer 4META had to be reduced to 3 wt% as opposed to 5 wt% used initially. Active composites with remineralising (MCPM) and antibacterial (PLS) were then prepared. The ratio of bulk to diluent monomer was kept at 3:1 to avoid using excessive diluent monomer, which may result in polymerisation shrinkage, however, very low levels of the monomers will compromise polymerisation. In addition, higher viscosity reduces sedimentation of particles that may be particularly important at low PLR.

The experiments

4.1 Visual assessment of monomers

All monomers were visually inspected after mixing, to investigate any residual solid monomers that failed to dissolve. All monomers containing 5 wt% 4META required other monomers such as DMAEMA to improve dissolution.

It is important that monomers are clear and solid monomers are fully dissolved. This ensures uniform monomer conversion. Solid particles will cause scattering affecting clarity and causing background scattering in UV spectra. Light, needs to be absorbed by the initiator to start the polymerization process.

Formulations A1 and B1 contained 0% DMAEMA and were both cloudy with visible solid particles most likely to be 4META. However, it was noticed that B1 became clear after it was left for 24 hours due to precipitation of the undissolved 4META, this probably indicates a reaction between TEGDMA and 4META or it is probably due to TEGDMA being less viscous than PPGDMA as a similar observation was not seen with A1. The colour of the all the monomers was yellow due to CQ.

Formulations of group C contained different amounts of CQ (0.5 and 1 wt%) and visual inspection did not detect any difference in the depth of colour; this was the same for formulations in group D that contained 0.75 or 1 wt% of CQ.

4.2 UV spectra of monomers

UV spectrometry was used to assess the clarity of the monomers containing different percentages of activator and initiator. Since the colour of the monomer is yellow; due to CQ peak absorbance was at wavelength of 470 mm the wavelength of blue light; hence the LED colour of the light-curing unit is blue. The difference in absorbance was affected by the amount of CQ only; therefore there was superimposition of the graph lines Figure 3.2 to 3.4. Figure 3.2 represents monomer groups A and B. Monomers containing 0 wt% DMAEMA were cloudy A1 and B1, the cloudiness of A1 explained by the high background scatter, as the graph appears higher despite containing a similar amount of CQ wt%. On the other hand, B1 monomer is detected by the UV spectrometry machine as being clear although when visually inspected it was cloudy. This is due to participation of the solid monomer 5 wt% 4META in B1 a TEGDMA monomer, with 0 wt% DMAEMA, probably due to low viscosity of (TEGDMA). The solid particles were noticeable on the floor of the container. All of the other monomers in groups A and B were clear and since they contained the same amount of CQ wt% the absorbance values were the same.

Series C monomers UV results are shown in figure 3-3, where the wt% of DMEMA was kept constant at 1 wt%, whereas the CQ wt% was variable i.e. 0.5 and 1 wt%. Peak absorbance was at wavelength of 470 as expected from the colour of the monomer. The difference in absorbance was affected by modifying the amount of CQ as there was superimposition of the graphs (C1 with C3 and C2 with C4) refer to table 3-4 for formulations.

The last figure on absorbance Figure 3.4 represents group D monomers that either contained bulk (UDMA) with or without diluent monomer (PPGDMA) and two different wt% of CQ (0.75 and 1). In general absorbance of monomer liquids containing UDMA only and no diluent monomer is slightly higher probably indicating slight cloudiness, than those containing a diluent monomer.

It is clear that monomers with 1 wt% of CQ are more concentrated, as absorbance is higher at a wavelength of 470 ηm, signifying a darker yellow colour Figures 3.3, 3.4 and 3.5.

A linear relationship exists between the amount of CQ wt% and absorbance at 470 (nm) as can be seen in figure 3.5 and expected from the Beer Lambert law. Absorbance reached a peak at 470 (nm) due to CQ that is yellow in colour.

Intrinsic factors effecting composite colour (factors within the composite)

The colour of a composite resin filling material can be affected by intrinsic factors i.e. factors within the material itself or extrinsic factors such as food. It is known that within a composite system, usually it is required that an activator and an initiator are used in order to maximise the polymerisation reaction.

It is essential that an optimum amount of CQ be used for a maximum MC to insure colour stability. This is because, the change on the colour of a restoration is often attributed to residual unreacted monomers, which result in the polymer being prone to oxidising reactions and therefore, material discolouration (Schroeder and Vallo, 2007).

In the first phase of this thesis, the activator being tested was DMAEMA combined with CQ at a constant concentration initially (0.75 wt%), in order to determine its effect on the colour and clarity of the monomer phase first. Since absorbance of CQ is known, it was easy to determine its effect on the colour of the monomer by spectrometry.

Both monomer formula A1 and B1 in the first experiment had 0 wt% DMAEMA, and visually they looked cloudy. This is most probably because of 4META, a solid monomer that can be difficult to dissolve.

The degree of conversion is directly related to mechanical properties such as flexure strength (Lovell et al., 2003) and will affect restoration longevity. Several factors affect

polymerisation, the type of monomer used one of the main ones. For example, using a flexible diluent monomer such as PPGDMA will result in more cross-linking. Adding to that, monomers with high molecular mass are more likely to result in an improved polymerisation, and so they are more biocompatible.

In the first set of monomer mixes, the aim was to compare the effect of changing the wt% of the activator DMAEMA on the clarity of the monomers and on MC%. Three different percentages of DMAEMA were used, it was noted that DMAEMA helped in clarifying monomers but did not result in an increased monomer conversion. In a recent article, it was stated that aliphatic amines such as DMAEMA have a reduced ability to react compared to others and this may affect monomer conversion (De Oliveira et al., 2015), thus it was omitted in later monomer phases where a reduced amount of 4META was used and the monomers were clear.

CQ has been used for many years as a photoinitiator (Albuqquerque et al., 2013), and it is preferred over previous initiators that use to depend on ultraviolet light curing (Stanbury, 2000). The main disadvantage of CQ is its bright yellow colour that inevitably affects the colour of the final material.

CQ was used at different concentrations for the initial experiments.It is normally used in small concentrations as it has a significant influence on the colour of the final material (Luiz et al., 2007).

4.3 FTIR analysis of composites

4.3.1 Reaction rates

Reaction kinetics are important as physical, biological and mechanical properties may be affected (Khan, 2015). Several factors may affect the polymerisation reaction and kinetics of composite materials; these include the type of monomers used as well as the amount of activator and initiator concentrations (Pomrink et al., 2003, Khan, 2015). In addition flexibility of the monomers and filler types used in a composite system may play an additional part (Pomrink et al., 2003).

Figures 4.7 to 4.9 demonstrate maximum reaction rates obtained from the FTIR of groups A, B, C and D3 with and without PLS. Figure 4.7 shows that reaction rates for group A i.e. monomers containing UDMA/PPGDMA decreased with increased levels of DMAEMA whereas the opposite is observed for group B UDMA/TEGDMA monomers, with maximum rate of 5.07 (5/s) is achieved with formulation B3 that contained 1.5 wt% (DMAEMA). On the other hand, figure 4.8; show that reaction rates increased with lower CQ wt% for both PPGDMA and TEGDMA monomers. PLS concentration seems to not affect reaction rates.

4.3.2 The degree of conversion:

In a resin composite restoration, the degree of monomer conversion is important, as this will affect its mechanical properties as well as biocompatibility and colour stability (Schroeder and Vallo, 2007, Demarco et al., 2012). Research showed that failure and fracture of composites is attributed to weak mechanical properties of composites. Several factors can affect monomer conversion in a composite system, including the monomers used; light curing time and light strength (Lui et al., 2013), as previously mentioned.

Monomer conversion is also important in determination of mechanical properties (Walters et al., 2016, Demarco et al., 2012). Ideally, once a composite material is cured no contents should leach out of it. FTIR was used to determine the MC%. It enables the actual polymerisation to be measured (Ilie et al., 2014) and it is relatively straightforward (Shin et al., 2009). Beside improved mechanical properties, a high monomer conversion is said to result in increased colour stability (Shin et al., 2009).

Generally, Composite containing UDMA as a bulk monomer, are thought to have a higher conversion, as UDMA is an aliphatic monomer and more flexible than Bis-GMA (Sideridou et al., 2002, Liaqat, 2015) and this was also true for the results in this project.

Monomer conversion was checked for formulation groups A, B, C and D, all contained UDMA and PPGDMA or TEGDMA. The ratio of UDMA (the bulk monomer) to either TEGDMA or PPGDMA (the diluent monomers) was 3:1, see table 3.4.

In this study, high monomer conversion was achieved with composites containing UDMA and PPGDMA with statistically significant results $P<0.001$. This is probably due to PPGDMA being a large and flexible molecule, which has fewer carbon-carbon double bonds (Walters et al, 2016).

For group A, monomer conversion was almost 90% as shown in figure 4.7 which is much higher than some commercial dental composites (Liaqat, 2015). There was no big difference in monomer conversion between monomers containing 0 wt% DMAEMA and those containing 1 to 1.5 wt%, probably indicating that DMAEMA is not affecting the conversion in monomers containing UDMA as a bulk monomer and PPGDMA as a diluent monomer. Monomer conversion for A1 was about 87% and was 86% and 85% for A2 and A3 respectively.

Formulation	CQ wt%	DMAEMA wt%	MC%
A2	0.75	1	**86.3**
C3	1	1	72.8
C4	0.5	1	78.3
B2	0.75	1	69.4
C1	1	1	64.9
C2	0.75	1	**68.6**

Table 5-1 The relationship between the wt% of CQ and MC% in formulations containing UDMA/PPGDMA (A2, C3 and C4), and formulations containing UDMA/TEGDMA (B2, C1 and C2).

4.4 The effect of changing PLR on handling

Using D3 as a liquid phase, two formulations were made with hybrid glass and two concentrations of MCPM and PLS see table 3-6 above. Formulations were produced at three different PLRs (3,4 and 5). Due to higher liquid content in formulations of PLR 3, composites were softer and the pastes were sticky. On the other hand, composites of PLR 5 were hard due to high powder content. Lower PLR improves flow and length of resin tags that enable bonding (Alkhouri, unpublished).

4.5 Mass and volume change and water sorption

Composite materials that are resin based are known for their potential of attracting water through the process of water sorption by diffusion (Oe Rtengren et al., 2001). Water sorption affects mechanical properties as well as the colour of a material (Arocha et al., 2014). It is largely depending on the type of resins used within a composite system, the cross linking level and the type of fillers (Sideridou et al., 2008). Composites containing MCPM encourage water sorption and provide a source of Calcium and Phosphate ions to promote remineralisation and this is in agreement with previous EDI projects on novel composites. Water sorption can be beneficial if balanced against polymerisation shrinkage.

Archimedes theory is a commonly used method of evaluating volumetric expansion (Ruttermann et al., 2011) and it was used to evaluate water sorption in several previous EDI projects and the current project.

Samples were stored in water for a period of eight weeks in order to check water sorption in the different formulations. From figure 4.10 it can be seen that % mass change was significant during the first week in formulations F1 and F2 with the different PLR (3, 4 and 5). In previous EDI projects mass change has been attributed to water sorption process in formulations containing MCPM (Mehdawi et al., 2009, Liaqat, 2015,

Ben Nuba, 2016). However, formulations containing a higher amount of polylysine had the highest mass change, probably due to it being a hydrophilic agent.
The highest % mass change was achieved by F1/3 about 4.08 with PLR 3 and 4 and 5 wt% MCPM and PLS respectively.

4.6 Water sorption and solubility

Solubility of particles was calculated from water sorption. The highest solubility was achieved with formulations F1/3 and F1/4 at eight weeks probably due to higher water sorption, which encouraged particle solubility. F1 formulations (see table 3-6, for formulations) contained 8 wt% MCPM which is hydrophilic.

4.7 Cumulative polylysine (PLS) release

PLS 2 and 5 wt% were used as they were both found to be effective on low bacterial counts (Lygidakis, unpublished). Higher PLS release was achieved in formulations containing higher PLS wt%. In addition, formulations with higher powder content contain higher amounts of PLS and this resulted in an increased release.

Composite discs colour evaluation

Extrinsic factors affecting composite colour

Visual assessment

It is important to be able to predict the colour stability of a filling material, as it is one of the causes of composite failure (Arocha et al., 2014). Factors affecting colour extrinsically can be affected by the presence of stains from oral hygiene products, food products, water sorption and surface texture (Nakazwa, 2009, Arocha 2014).
In these colour evaluation experiments, discs from the different formulations of the novel composites were immersed in commonly consumed solutions and commonly used in colour evaluation experiments.

4.8 Spectroshade assessment of colour change

Results are given in section 3.9; in general red wine resulted in significant discolouration in all the tested formulations. Formulations with PLR 5 had the highest ΔE values indicating more change in colour in all the three solutions; this may be attributed to the high powder content that resulted in surface roughness of the material (Yu et al, 2009). There was no big difference in formulations of PLR 3 and 4. In general values of ΔE that are less than 3.3 are unnoticeable (Shamszadeh et al., 2016), Figure 3.23 present the results obtained for experimented composites, it is clear

that the lowest discolouration was with discs immersed in coke and the values were close to 3.3 and the discolouration was not detected by direct vision see table 3-5, section 3.8.

Comparing the tested composites and the commercial materials, Fgures 4.21 and 4.22), commercial composite discs were similarly affected with values of ΔE ranging from 3.6(in coke) to (18.5 in red wine).

Chapter Five

Conclusion and future work

5 Conclusion and future work

5.1 Conclusion

This project investigated several factors in producing novel composite restorative materials, for example the possibility of replacing commonly used bulk BisGMA with (UDMA) and the diluent monomer TEGDMA with PPGDMA and in the liquid phase. It can be concluded that it is possible to use CQ (a common photoinitiator) without an activator such as DMAEMA. However, where other solid monomers are used; e.g. 4META, a small amount of DMAEMA can help produce clear monomers.

In terms of MC%, monomers containing PPGDMA had the highest conversion with or without an initiator. Reaction rates for monomers containing PPGDMA and DMAEMA as an initiator were inversely proportional to MC% whereas the opposite was true for TEGDMA monomers (see figure 3.7).

Mass and volume changes of the novel composites in water were much higher than those of the commercial composite. Formulation one of PLR (3, 4 AND 5) had the highest mass change and the highest water sorption.

Formulations with PLR 5 were highly discoloured by the different solutions.

5.2 Future work

Monomer conversion of formulations F1 and F2

The current study mainly evaluated the effect of modifying the monomer phase on the conversion; future studies should also evaluate the effect of the powder phase on conversion, in particular composites containing MCPM.

Monomer conversion versus depth of cure

The current study evaluated the monomer conversion at 1mm depth, more studies are necessary to evaluate if the monomer conversion of composite containing PPGDMA at larger depths is affected.

In vitro studies in the human pulp

In vitro studies to evaluate the effect of the novel materials on pulp cells to assess biocompatibility would be useful, although these will be limited by not mimicking the actual clinical situation

Clinical evaluation of the final material

Although the current study provides a preliminary evaluation of colour stability, clinical trials maybe necessary to provide an in-vivo evaluation of aesthetics. Adding to that, clinical trials may provide a means of assessing other properties such as longevity of the new material and adhesion and probably biocompatibility.

Adhesion properties

An adhesion promoting monomer (4META) has been included into the novel composites; therefore it will be interesting to investigate the adhesion properties using human teeth. Several tests can be used e.g. the push out test and the shear test.

Antibacterial studies

Clinical studies to assess antibacterial effects on carious teeth and biofilm may provide a clearer picture on the accurate concentration of the antibacterial to be used

Chapter Six

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7 Appendices

7.1 Statistics FTIR

Between-Subjects Factors

		Value Label	N
AorB	1	A	9
	2	B	9

Tests of Between-Subjects Effects

Dependent Variable: MC_percent

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1251.157[a]	2	625.578	123.195	.000
Intercept	34686.920	1	34686.920	6830.861	.000
concentration (CQ)	40.017	1	40.017	7.880	.013
AorB	1211.140	1	1211.140	238.509	.000
Error	76.170	15	5.078		
Total	103282.048	18			
Corrected Total	1327.326	17			

a. R Squared = .943 (Adjusted R Squared = .935)

7.2 Mass % change

7.2.1 Univariate Analysis of Variance

Between-Subjects Factors

		N
formulation	1.00	27
	2.00	27
ratio	3.00	18
	4.00	18
	5.00	18

Tests of Between-Subjects Effects

Dependent Variable: **mass_raw**

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	56.092[a]	5	11.218	81.312	.000
Intercept	357.874	1	357.874	2593.904	.000
formulation	46.212	1	46.212	334.946	.000
ratio	8.000	2	4.000	28.991	.000
formulation * ratio	1.881	2	.940	6.817	.002
Error	6.622	48	.138		
Total	420.588	54			
Corrected Total	62.715	53			

a. R Squared = .894 (Adjusted R Squared = .883)

7.3 Volume % change

7.3.1 univariate Analysis of Variance

Between-Subjects Factors

		N
formulation	1.00	27
	2.00	27
ratio	3.00	18
	4.00	18
	5.00	18

Tests of Between-Subjects Effects

Dependent Variable: volume_raw

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.656[a]	5	.731	1.801	.131
Intercept	155.506	1	155.506	383.010	.000
formulation	.460	1	.460	1.132	.293
ratio	2.673	2	1.337	3.292	.046
formulation * ratio	.523	2	.261	.644	.530
Error	19.489	48	.406		
Total	178.651	54			
Corrected Total	23.145	53			

a. R Squared = .158 (Adjusted R Squared = .070)

7.4 Effect of immersion of composite discs in different solutions

Coke

(I) composition	(J) composition	Sig.
f1/3	f1/4	.190
	f1/5	1.000
	f2/3	1.000
	f2/4	1.000
	f2/5	.636
f1/4	f1/5	.051
	f2/3	1.000
	f2/4	.120
	f2/5	1.000
f1/5	F2/3	1.000
	f2/4	1.000
	f2/5	.172
f2/3	F2/4	1.000
	f2/5	1.000
f2/4	F2/5	.404

Based on observed means.

The error term is Mean Square(Error) = .387.

Redwine

(I) composition	(J) composition	Sig.
f1/3	f1/4	1.000
	f1/5	1.000
	f2/3	1.000
	f2/4	1.000
	f2/5	1.000
f1/4	f1/5	1.000
	f2/3	1.000
	f2/4	1.000
	f2/5	1.000
f1/5	F2/3	1.000
	f2/4	1.000
	f2/5	1.000
f2/3	F2/4	1.000
	f2/5	1.000
f2/4	F2/5	1.000

Based on observed means.

The error term is Mean Square(Error) = 14.573.

Tea

(I) composition	(J) composition	Sig.
f1/3	f1/4	.196
	f1/5	.000
	f2/3	.008
	f2/4	.002
	f2/5	.000
f1/4	f1/5	.049
	f2/3	1.000
	f2/4	.397
	f2/5	.027
f1/5	F2/3	1.000
	f2/4	1.000
	f2/5	1.000
f2/3	F2/4	1.000
	f2/5	.717
f2/4	F2/5	1.000

Based on observed means.

The error term is Mean Square(Error) = 1.306.

7.5 Readings from the spectroshade for F1 and F2, PLR 3

F1/3 (reference)	Coke point (1)	ΔE
Shade B1 L 76.46 C 3.77 H 103.58	L 78.83 C 6.73 H 97.28	3.83
	Point (2) L 78.05 C 7.25 H 101.3	3.82
	Point (3) L 78.82 C 6.81 H 7.04	3.9
ΔE		

F1/3 (reference)	Tea point (1)	ΔE
Shade B1 L 76.45 C 3.78 H 103.1	L 73.72 C 6.3 H 101.86	3.72
	Point (2) L 72.08 C 6.97 H 104.69	5.41
	Point (3) L 72.75 C 6.15 H 91.88	4.49
ΔE		

F1/3 (reference)	Red wine (1)	ΔE
Shade B1 L 76.5 C 3.8 H 102.33	L 68.52 C 12.19 H 94.27	11.62
	Point (2) L 66.09 C 11.3 H 97.19	12.85
	Point (3) L 65.87 C 11.49 H 83.45	13.3
ΔE		

F2/3 (reference)	Coke point (1)	ΔE
Shade B1 L 76.28 C 0.89 H 130.8	L 78.11 C 5.24 H 93.71	4.9
	Point (2) L 75.48 C 4.5 H 97.36	3.87
	Point (3) L 75.16 C 4.88 H 80.21	4.61
ΔE		

F2/3 (reference)	Tea point (1)	ΔE
Shade B1 L 76.35 C 0.85 H 133.59	L 76.72 C 7.52 H 101.14	6.83
	Point (2) L 72.25 C 8.65 H 103.45	8.92
	Point (3) L 72.5 C 8.65 H 103.45	8.92
ΔE		

F2/3 (reference)	Red wine (1)	ΔE
Shade B1 L 76.49 C 0.85 H 130.29	L 68.52 C 10.48 H 91.44	12.65
	Point (2) L 64.98 C 10.45 H 94.43	15.1
	Point (3) L 65.9 C 10.48 H 8.28	14.53
ΔE		

7.6 Readings from the spectroshade for F1 and F2, PLR 4

F1/4 (reference)	Coke (1)	ΔE
Shade B1 L 79.94 C 4.01 H 105.99	L 79.71 C 9.1 H 93.92	5.25
	Point (2) L 77.89 C 8.37 H 95.6	4.93
	Point (3) L 77.06 C 8.7 H 98.714	5.83
ΔE		

F1/4(reference)	Tea (1)	ΔE
Shade B1 L 79.93 C 4 H 105.86	L 78.71 C 9.81 H 97.82	6
	Point (2) L 75.92 C 9.09 H 89.28	6.71
	Point (3) L 74.63 C 8.53 H 100.18	6.99
ΔE		

F1/4 (reference)	Red wine (1)	ΔE
Shade B1 L 79.95 C 4 H 105.7	L 74.42 C 13.75 H 90.02	11.39
	Point (2) L 71.26 C 11.55 H 93.1	11.60
	Point (3) L 71.84 C 12.33 H 86.01	11.87
ΔE		

F2/4 (reference)	Coke (1)	ΔE
Shade B1 L 78.99 C 1.7 H 106.79	L 79.43 C 5.58 H 97.31	3.93
	Point (2) L 77.5 C 4.29 H 97.37	3.02
	Point (3) L 77.1 C 5.32 H 86.08	4.22
ΔE		

F2/4 (reference)	Tea (1)	ΔE
Shade B1 L 79.03 C 1.77 H 101.48	L 73.41 C 6.01 H 102.67	7.04
	Point (2) L 69.7 C 6.44 H 104.04	10.44
	Point (3) L 70.54 C 5.46 H 86.21	9.30
ΔE		

F2/4 (reference)	Red wine(1)	ΔE
Shade B1 L 78.98 C 1.72 H 104.43	L 72.53 C 8.57 H 93.45	9.43
	Point (2) L 68.38 C 7.03 H 94.12	11.86
	Point (3) L 69.37 C 8.16 H 82.94	11.65
ΔE		

7.7 Readings from the spectroshade for F1 and F2, PLR 5

F1/5 (reference)	Coke (1)	ΔE
Shade B1 L 81.24 C 3.8 H 105.5	L 79.8 C 5.47 H 94.87	2.36
	Point (2) L 77.66 C 4.51 H 91.87	3.78
	Point (3) L 77.58 C 5.63 H 88.01	4.33
ΔE		

F1/5 (reference)	Tea(1)	ΔE
Shade B1 L 81.32 C 3.82 H 105.36	L 76.79 C 11.56 H 94.51	9.05
	Point (2) L 72.43 C 11.32 H 92.9	11.72
	Point (3) L 75.6 C 10.79 H 89.92	9.17
ΔE		

F1/5 (reference)	Red wine (1)	ΔE
Shade B1 L 81.28 C 3.8 H 105.4	L 75.85 C 12.65 H 8.76	10.65
	Point (2) L 72.39 C 11.36 H 85.8	11.88
	Point (3) L 72.88 C 13.65 H 78.54	26.86
ΔE		

F2/5 (reference)	Coke (1)	ΔE
Shade B1 L 82.18 C 2.51 H 116.24	L 80.51 C 6.28 H 96.3	4.35
	Point (2) L 77.48 C 5.41 H 99.62	5.62
	Point (3) L 78.57 C 5.54 H 88.53	5.04
ΔE		

F2/5(reference)	Tea (1)	ΔE
Shade B1 L 82.39 C 2.5 H 116.08	L 75.43 C 8.59 H 93.18	9.43
	Point (2) L 72.12 C 6.79 H 92.35	11.26
	Point (3) L 73.95 C 7.73 H 87.82	10.15
ΔE		

F2/5(reference)	Red wine (1)	ΔE
Shade B1 L 82.29 C 2.52 H 116.45	L 70.71 C 9.02 H 84.97	13.52
	Point (2) L 67.91 C 8.38 H 88.96	15.68
	Point (3) L 68.02 C 8.7 H 75.57	15.89
ΔE		

7.8 Z250 Readings

Z250 (reference)	Coke (1)	ΔE
Shade B1 L 72.49 C .51 H 296.6	L 69.96 C 1.7 H 101.26	3.35
	Point (2) L 66.78 C 0.74 H 191.82	3.34
	Point (3) L 64.90 C 2.46 H 324.94.	7.85
ΔE		

F2/5(reference)	Tea (1)	ΔE
Shade B1 L 72.49 C .51 H 296.6	L 69.99 C 6.87 H 99.95	7.77
	Point (2) L 67.4 C 5.11 H 110.61	7.58
	Point (3) L 67.41 C 4.46 H 88.1	7.07
ΔE		

F2/5(reference)	Red wine (1)	ΔE
Shade B1 L 72.49 C .51 H 296.6	L 67.95 C17.99 H 90.67	19
	Point (2) L 63.77 C 15.56 H 95.08	18.26
	Point (3) L 66.58 C 16.78 H 85.04	18.2
ΔE		